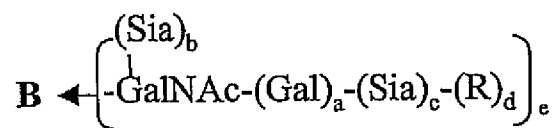B ← [-GalNAc-(Gal)$_a$-(Sia)$_c$-(R)$_d$]$_e$
      |
      (Sia)$_b$

a-c, e (independently selected) = 0 or 1;
d = 0;
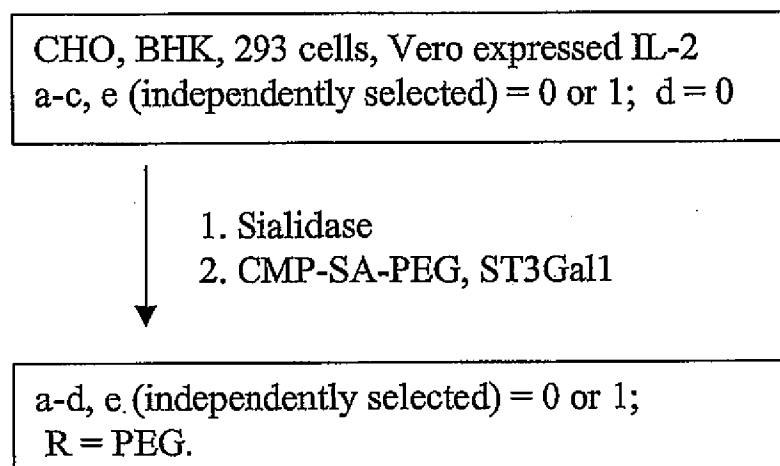R = modifying group, mannose, oligo-
mannose.

FIG. 39A

CHO, BHK, 293 cells, Vero expressed IL-2
a-c, e (independently selected) = 0 or 1; d = 0

1. Sialidase
2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1;
R = PEG.

FIG. 39B

Insect cell expressed IL-2
a, e (independently selected) = 0 or 1;
b, c, d = 0.

1. Galactosyltransferase, UDP-Gal
2. CMP-SA-PEG, ST3Gal1

a, c, d, e (independently selected) = 0 or 1;
R = PEG.

FIG. 39C

E. coli expressed IL-2
a-e = 0.

1. GalNAc Transferase, UDP-GalNAc
2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1;
a, b = 0; R = PEG.

FIG. 39D

NSO expressed IL-2
a, e (independently selected) = 0 or 1;
b, c, d = 0

1. CMP-SA-levulinate, ST3Gal1
2. H_4N_2-PEG

a, c, d, e (independently selected) = 0 or 1;
b = 0; R = PEG.

FIG. 39E

E. coli expressed IL-2
a-e = 0.

1. Endo-GalNAc-osaminidase
   (synthetic enzyme),
   PEG-Gal-GalNAc-F

a, d, e = 1; b, c = 0;
R = PEG.

FIG. 39F

E. coli expressed IL-2
a-e = 0.

1. GalNAc Transferase,
   UDP-GalNAc-PEG

d, e = 0 or 1; a,-c = 0;
R = PEG.

FIG. 39G

2 peptides
A and A' - N-linked sites
B - O-linked sites
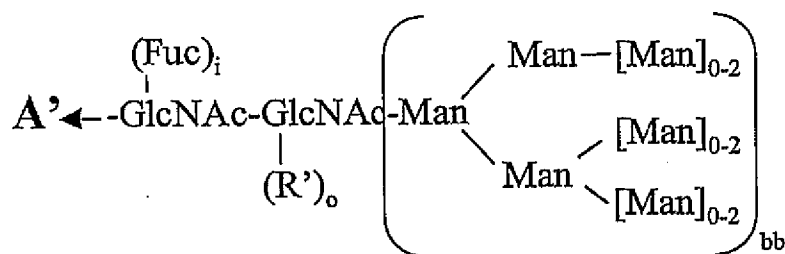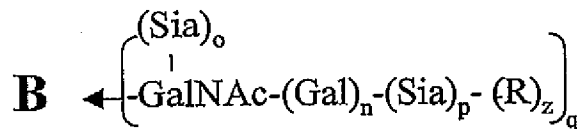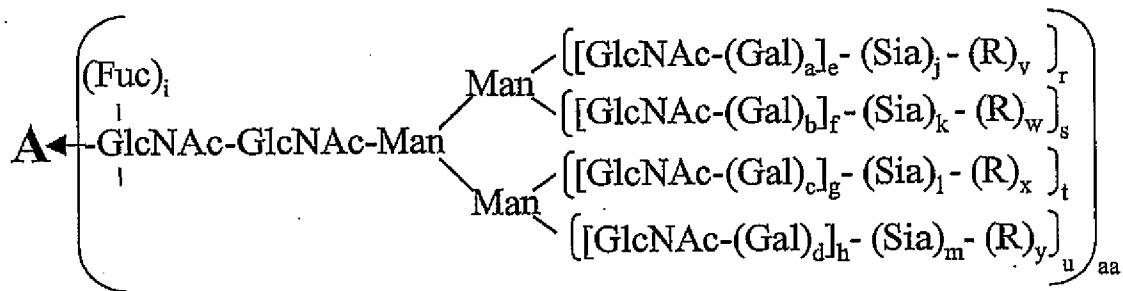
$$\mathbf{A} \longleftarrow \left(\begin{array}{c} \text{(Fuc)}_i \\ | \\ \text{-GlcNAc-GlcNAc-Man} \\ | \end{array} \right. \begin{array}{c} \text{Man} \Big\langle \begin{array}{c} \big[[\text{GlcNAc-(Gal)}_a]_e - \text{(Sia)}_j - \text{(R)}_v \big]_r \\ \big[[\text{GlcNAc-(Gal)}_b]_f - \text{(Sia)}_k - \text{(R)}_w \big]_s \end{array} \\ \text{Man} \Big\langle \begin{array}{c} \big[[\text{GlcNAc-(Gal)}_c]_g - \text{(Sia)}_l - \text{(R)}_x \big]_t \\ \big[[\text{GlcNAc-(Gal)}_d]_h - \text{(Sia)}_m - \text{(R)}_y \big]_u \end{array} \right)_{aa}$$

$$\mathbf{B} \longleftarrow \left[\begin{array}{c} \text{(Sia)}_o \\ | \\ \text{-GalNAc-(Gal)}_n\text{-(Sia)}_p - \text{(R)}_z \end{array} \right]_q$$

$$\mathbf{A'} \longleftarrow \begin{array}{c} \text{(Fuc)}_i \\ | \\ \text{-GlcNAc-GlcNAc-Man} \\ | \\ \text{(R')}_o \end{array} \left(\begin{array}{c} \text{Man} - [\text{Man}]_{0-2} \\ \text{Man} \Big\langle \begin{array}{c} [\text{Man}]_{0-2} \\ [\text{Man}]_{0-2} \end{array} \end{array} \right)_{bb}$$

Alternate structure
for some N-linked
structures of A.

a-d, i, n-u (independently selected) = 0 or 1.
aa, bb (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 20.
v-z = 0; R = polymer, glycoconjugate.

FIG. 40A

CHO, BHK, 293s cells, Vero, MDCK, HEKC expressed
    Factor VIII.
e-h = 1 to 4;
aa, bb, a-d, j-m, i, n-u (independently selected) = 0 or 1;
v-z = 0.

      ↓ 1. Sialidase
      ▼ 2. CMP-SA-PEG, ST3Gal3

e-h = 1 to 4;
aa, bb, a-d, i, n, q-u (independently selected) = 0 or 1;
o, p, z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 40B

CHO, BHK, 293S cells, Vero, MDCK, 293S, HEKC
    expressed Factor VIII.
e-h = 1 to 4;
aa, bb, a-d, j-m, i, n-u (independently selected) = 0 or 1;
v-z = 0.

      ↓ 1. Sialidase
         2. CMP-SA-PEG, ST3Gal3
      ▼ 3. ST3Gal1, CMP-SA

e-h = 1 to 4;
aa, bb, a-d, i, n, p-u (independently selected) = 0 or 1;
o, z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 40C

CHO, BHK, 293s cells, Vero, MDCK, HEKC
    expressed Factor VIII.
e-h = 1 to 4;
aa, bb, a-d, j-m, i, n-u (independently selected)=0 or 1;
v-z = 0.

1. CMP-SA-PEG, ST3Gal3

e-h = 1 to 4;
aa, bb, a-d, i, n-u (independently selected) = 0 or 1;
z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 40D

CHO, BHK, 293S cells, Vero, MDCK, HEKC
    expressed Factor VIII.
e-h = 1 to 4;
aa, bb, a-d, j-m, i, n-u (independently selected) 0 or 1;
v-z = 0.

1. CMP-SA-PEG, ST3Gal1

e-h = 1 to 4;
aa, bb, a-d, i, n-u (independently selected) = 0 or 1;
z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 40E

CHO, BHK, 293S cells, Vero, MDCK, HEKC
   expressed Factor VIII.
e-h = 1 to 4;
aa, bb, a-d, j-m, i, n-u (independently selected)=0 or 1;
v-z = 0.

1. CMP-SA-PEG, α2,8-ST

e-h = 1 to 4;
aa, bb, a-d, i, n-y (independently selected) = 0 or 1;
z = 0; j-m (independently selected) = 0 to 2;
v-y (independently selected) = 1,
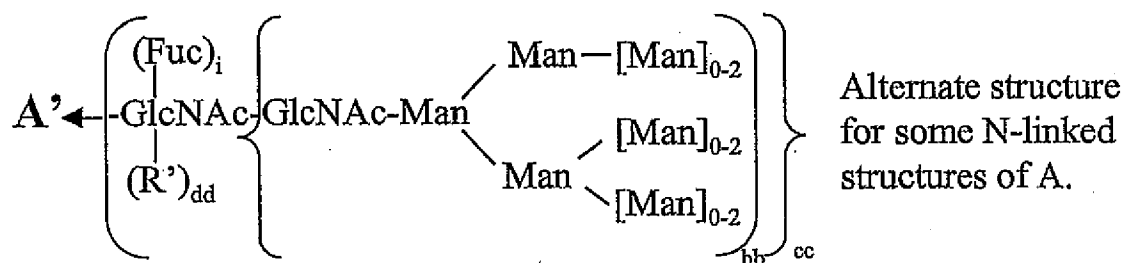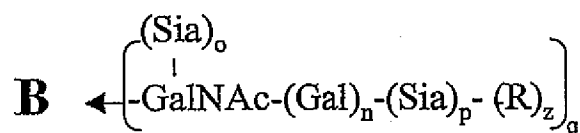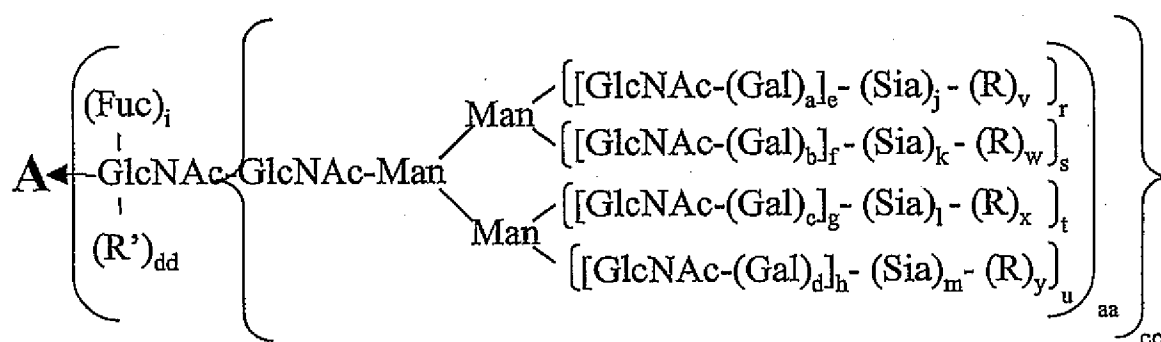   when j-m (independently selected) is 2;
R = PEG.

FIG. 40F

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$$
\boxed{
\begin{array}{l}
\text{2 peptides} \\
\mathbf{A\ or\ A'} \text{ - N-linked sites} \\
\mathbf{B} \text{ - O-linked sites}
\end{array}
}
$$

$$
\mathbf{A} \leftarrow \left(
\begin{array}{c}
(\text{Fuc})_i \\
| \\
\text{-GlcNAc-GlcNAc-Man} \\
| \\
(\text{R'})_{dd}
\end{array}
\right)
\left\{
\begin{array}{l}
\text{Man} \Big\langle
\begin{array}{l}
\big[[\text{GlcNAc-(Gal)}_a]_e\text{- (Sia)}_j\text{- (R)}_v\big]_r \\
\big[[\text{GlcNAc-(Gal)}_b]_f\text{- (Sia)}_k\text{- (R)}_w\big]_s
\end{array} \\
\text{Man} \Big\langle
\begin{array}{l}
\big[[\text{GlcNAc-(Gal)}_c]_g\text{- (Sia)}_l\text{- (R)}_x\big]_t \\
\big[[\text{GlcNAc-(Gal)}_d]_h\text{- (Sia)}_m\text{- (R)}_y\big]_u
\end{array}
\end{array}
\right\}_{aa} \right\}_{cc}
$$

$$
\mathbf{B} \leftarrow \left[
\begin{array}{c}
(\text{Sia})_o \\
| \\
\text{-GalNAc-(Gal)}_n\text{-(Sia)}_p\text{- (R)}_z
\end{array}
\right]_q
$$

$$
\mathbf{A'} \leftarrow \left(
\begin{array}{c}
(\text{Fuc})_i \\
| \\
\text{-GlcNAc-GlcNAc-Man} \\
| \\
(\text{R'})_{dd}
\end{array}
\right)
\left\{
\begin{array}{l}
\text{Man} - [\text{Man}]_{0-2} \\
\qquad [\text{Man}]_{0-2} \\
\text{Man} \Big\langle \\
\qquad [\text{Man}]_{0-2}
\end{array}
\right\}_{bb\ cc}
$$

Alternate structure
for some N-linked
structures of A.

a-d, i, n-u, (independently selected) = 0 or 1.
aa, bb, cc, dd (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 20.
v-z = 0;
R = modifying group, mannose, oligo-mannose.
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 40G

CHO, BHK, 293S cells, Vero, MDCK, HEKC
   expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.

        ↓   1. CMP-SA-levulinate, ST3Gal3,
            2. H_4N_2-PEG

e-h = 1 to 4;
aa, bb, cc, a-d, i, n-u (independently selected) = 0 or 1;
dd, z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 40H

CHO, BHK, 293S cells, Vero, MDCK, HEKC
   expressed Factor VIII.
e-h = 1 to 4;
 aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.

        ↓   1. endo-H
            2. galactosyltransferase, UDP-Gal-PEG

e-h = 1 to 4;
aa, bb, dd, a-d, i, j-u (independently selected) = 0 or 1;
cc, v-z = 0; R' = -Gal-PEG.

FIG. 40I

CHO, BHK, 293S cells, Vero, MDCK, HEKC
   expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.

    1. ST3Gal3, CMP-SA
↓ 2. endo-H
    3. galactosyltransferase, UDP-Gal-PEG

e-h = 1 to 4;
aa, bb, dd, a-d, i, j-u (independently selected) = 0 or 1;
cc, v-z = 0; R' = -Gal-PEG.

FIG. 40J

CHO, BHK, 293S cells, Vero, MDCK, HEKC
   expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.

    1. mannosidases
↓ 2. GNT 1 & 2, UDP-GlcNAc
    3. galactosyltransferase, UDP-Gal-PEG

e-h = 1 to 4;
aa, a-d, i, j-y (independently selected) = 0 or 1;
bb, cc, dd, z = 0; R = PEG.

FIG. 40K

CHO, BHK, 293S cells, Vero, MDCK, HEKC
   expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.

1. mannosidases
2. GNT-1,2, 4 & 5; UDP-GlcNAc
3. galactosyltransferase, UDP-Gal
4. ST3Gal3, CMP-SA

e-h = 1 to 4;
aa, bb, cc, a-d, i, j-q (independently selected) = 0 or 1;
dd, v-z = 0.

FIG. 40L

CHO, BHK, 293S cells, Vero, MDCK, HEKC
   expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
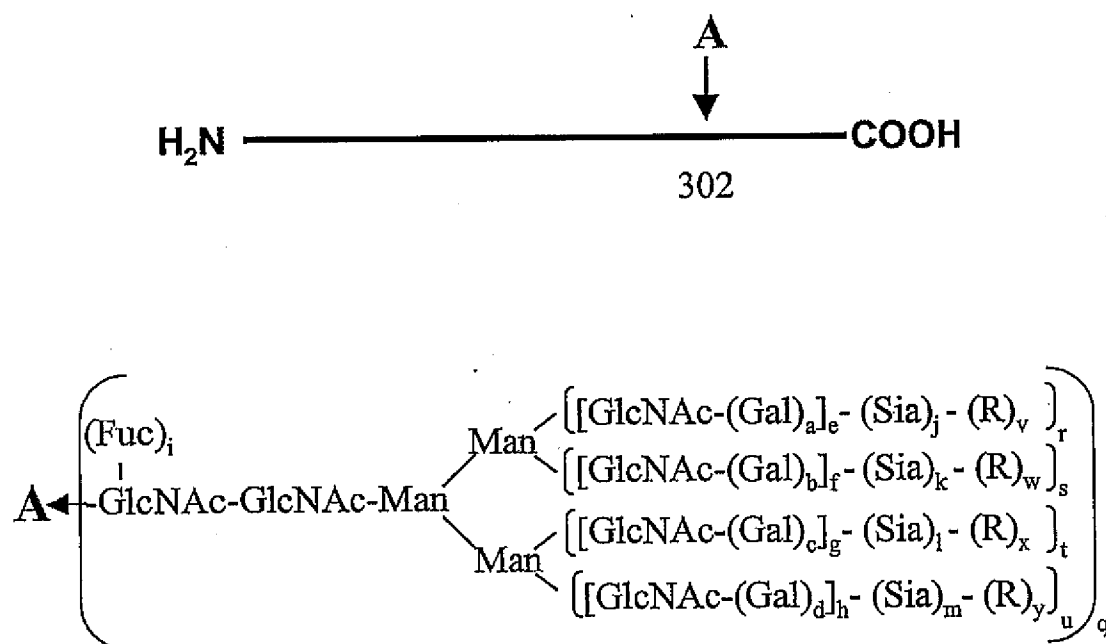dd, v-z = 0.

1. mannosidases
2. GNT-1, UDP-GlcNAc-PEG

e-h = 0 to 4;
aa, a-d, i, j-y (independently selected) = 0 or 1;
bb, cc, dd, z = 0.

FIG. 40M

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$$A$$
$$\downarrow$$

$$H_2N \text{———————————} COOH$$

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$$A \blacktriangleleft \left(\begin{array}{l} (Fuc)_i \\ | \\ \text{GlcNAc-GlcNAc-Man} \end{array} \right. \begin{array}{l} \text{Man}^{\diagup} \left[[GlcNAc\text{-}(Gal)_a]_e\text{-} (Sia)_j\text{-} (R)_v \right]_r \\ {}_{\diagdown} \left[[GlcNAc\text{-}(Gal)_b]_f\text{-} (Sia)_k\text{-} (R)_w \right]_s \\ \text{Man}^{\diagup} \left[[GlcNAc\text{-}(Gal)_c]_g\text{-} (Sia)_l\text{-} (R)_x \right]_t \\ {}_{\diagdown} \left[[GlcNAc\text{-}(Gal)_d]_h\text{-} (Sia)_m\text{-} (R)_y \right]_u \end{array} \left. \vphantom{\begin{array}{l} a \\ b \\ c \\ d \end{array}} \right)_q$$

a-d, i, q-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0; R = polymer.

FIG. 41A

```
┌─────────────────────────────────────────────────────┐
│ CHO, BHK, 293 cells, Vero expressed Urokinase.       │
│ a-d, i-m, q-u (independently selected) = 0 or 1;     │
│ e-h = 1; v-y = 0.                                    │
└─────────────────────────────────────────────────────┘
```

         1. Sialidase

         2. CMP-SA-PEG (16 mol eq),
            ST3Gal3

```
┌─────────────────────────────────────────────────────┐
│ a-d, i-m, q-u (independently selected) = 0 or 1;     │
│ e-h = 1; v-y (independently selected) = 1,           │
│    when j-m (independently selected) is 1;           │
│ R = PEG.                                             │
└─────────────────────────────────────────────────────┘
```

FIG. 41B

```
┌─────────────────────────────────────────────────────┐
│ CHO, BHK, 293 cells, Vero expressed Urokinase.       │
│ a-d, i-m, q-u (independently selected) = 0 or 1;     │
│ e-h = 1; v-y = 0.                                    │
└─────────────────────────────────────────────────────┘
```

         1. Sialidase

         2. CMP-SA-PEG (1.2 mol eq),
            ST3Gal3

         3. CMP-SA (16 mol eq), ST3Gal3

```
┌─────────────────────────────────────────────────────┐
│ a-d, i-m, q-u (independently selected) = 0 or 1;     │
│ e-h = 1; v-y (independently selected) = 0 or 1;      │
│ R = PEG.                                             │
└─────────────────────────────────────────────────────┘
```

FIG. 41C

NSO expressed Urokinase.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0;
Sia (independently selected) = Sia or Gal.

1. Sialidase and α-galactosidase
2. α-Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
   when j-m (independently selected) is 1;
R = PEG.

FIG. 41D

CHO, BHK, 293 cells, Vero expressed Urokinase.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. Sialidase
2. CMP-SA-PEG (16 mol eq),
   ST3Gal3
3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 41E

CHO, BHK, 293 cells, Vero expressed Urokinase.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA-levulinate, ST3Gal3,
   buffer, salt
2. H_4N_2-PEG

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
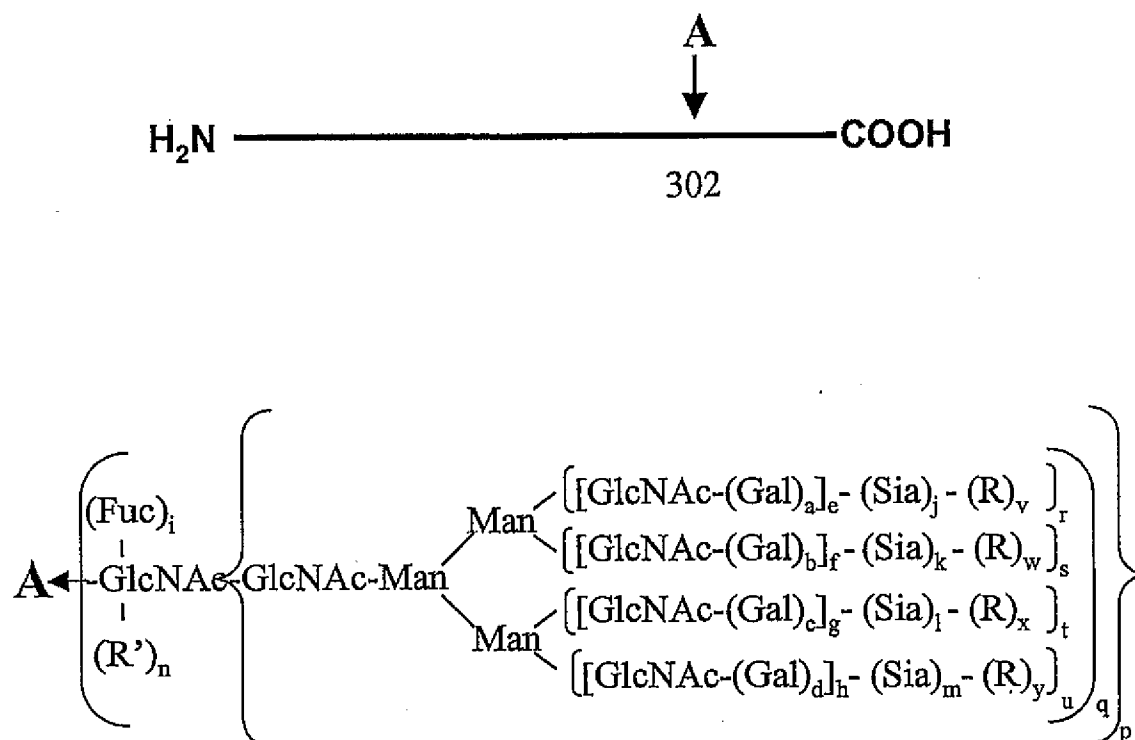R = PEG.
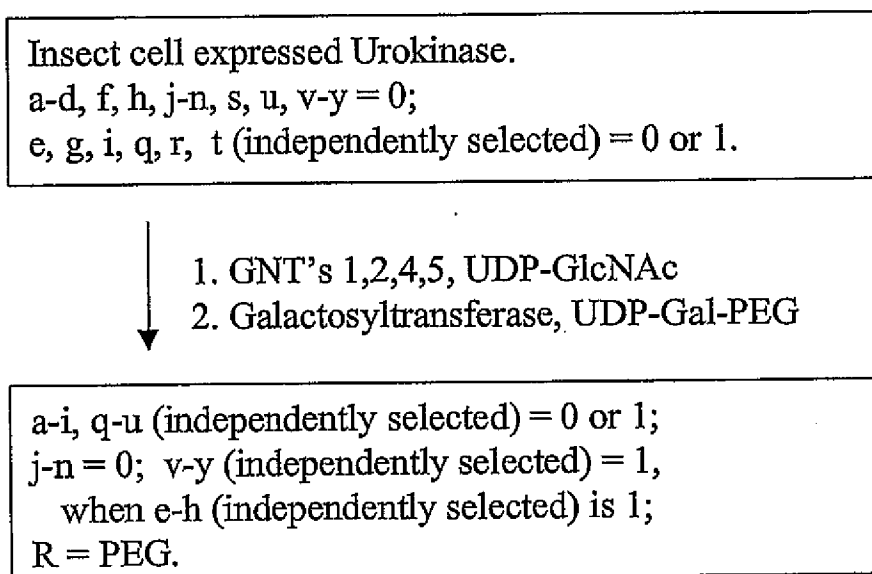
FIG. 41F

CHO, BHK, 293 cells, Vero expressed Urokinase.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1;
j-m (independently selected) = 0-20;
v-y (independently selected) = 0.

FIG. 41G

$$A$$

$$H_2N \underline{\hspace{4cm}} COOH$$

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$$A \blacktriangleleft \left[\begin{array}{c} (Fuc)_i \\ | \\ -GlcNAc \\ | \\ (R')_n \end{array} \right] \left\{ GlcNAc-Man \left\langle \begin{array}{c} Man \left\langle \begin{array}{c} \left[[GlcNAc-(Gal)_a]_e - (Sia)_j - (R)_v \right]_r \\ \left[[GlcNAc-(Gal)_b]_f - (Sia)_k - (R)_w \right]_s \end{array} \right. \\ Man \left\langle \begin{array}{c} \left[[GlcNAc-(Gal)_c]_g - (Sia)_l - (R)_x \right]_t \\ \left[[GlcNAc-(Gal)_d]_h - (Sia)_m - (R)_y \right]_u \end{array} \right. \end{array} \right\}_q \right\}_p$$

a-d, i, n, p-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose;
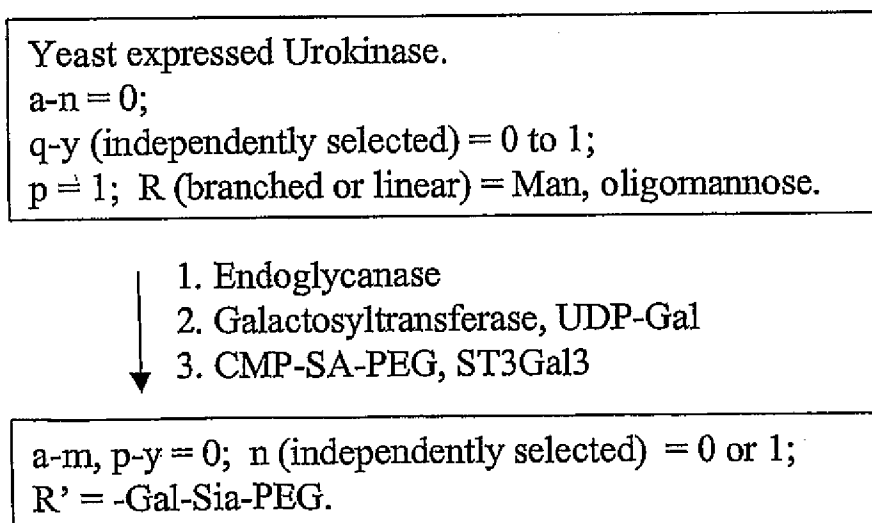R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 41H

Insect cell expressed Urokinase.
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.

1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1;
j-n = 0; v-y (independently selected) = 1,
   when e-h (independently selected) is 1;
R = PEG.

FIG. 41I

Yeast expressed Urokinase.
a-n = 0;
q-y (independently selected) = 0 to 1;
p = 1; R (branched or linear) = Man, oligomannose.

1. Endoglycanase
2. Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.

FIG. 41J

CHO, BHK, 293 cells, Vero expressed Urokinase.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; n, v-y = 0.

1. CMP-SA-linker-SA-CMP, ST3Gal3
2. ST3Gal1, desialylated Urokinase produced in CHO.
3. CMP-SA, ST3Gal3, ST3Gal1

a-m, q-u (independently selected) = 0 or 1;
p = 1; n = 0;
v-y (independently selected) = 0 or 1;
R = linker-Urokinase.

FIG. 41K

Isolated Urokinase.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0;   n = 0;
Sia (independently selected) = Sia or SO_4;
Gal (independently selected) = Gal or GalNAc;
GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc.

1. sulfohydrolase
2. CMP-SA-PEG, sialyltransferase

a-d, i-m, q-u (independently selected) = 0 or 1;
n = 0; e-h = 1; Sia = Sia;
Gal (independently selected) = Gal or GalNAc;
GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc.
 v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 41L

Isolated Urokinase.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; n = 0; v-y = 0;
Sia (independently selected) = Sia or SO_4;
Gal (independently selected) = Gal or GalNAc;
GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc.

1. sulfohydrolase, hexosaminidase
2. UDP-Gal-PEG, galactosyltransferase
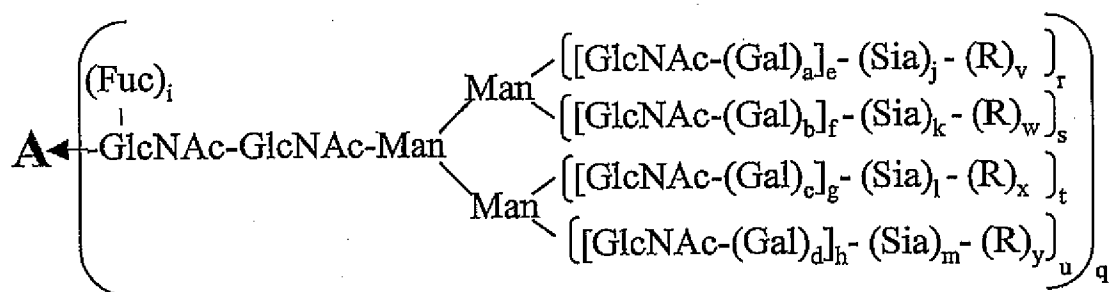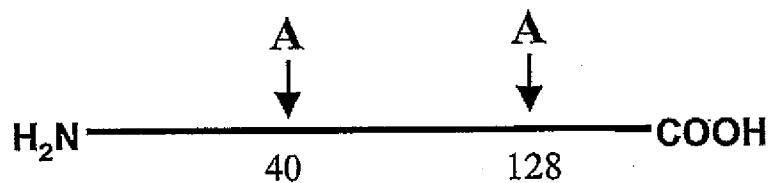
a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-n = 0; Gal (independently selected) = Gal;
GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc;
v-y (independently selected) = 0 or 1; R = PEG.

FIG. 41M

a-d, i, q-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0; R = polymer, glycoconjugate.

FIG. 42A

CHO, BHK, 293 cells, Vero expressed DNase I.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. Sialidase
2. CMP-SA-PEG (16 mol eq),
    ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
 e-h = 1;
 v-y (independently selected) = 1,
    when j-m (independently selected) is 1;
R = PEG.

FIG. 42B

CHO, BHK, 293 cells, Vero expressed DNase I.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. Sialidase
2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
 e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 42C

NSO expressed DNase I.
a-d, i-m, q-u (independently selected) = 0 or 1;
 e-h = 1; v-y = 0;
Sia (independently selected) = Sia or Gal.

1. Sialidase and α-galactosidase
2. α-Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
 e-h = 1; v-y (independently selected) = 1,
    when j-m (independently selected) is 1;
R = PEG.

FIG. 42D

CHO, BHK, 293 cells, Vero expressed DNase I.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. Sialidase
2. CMP-SA-PEG (16 mol eq), ST3Gal3
3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 42E

CHO, BHK, 293 cells, Vero expressed DNase I.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA-levulinate, ST3Gal3,
   buffer, salt
2. H_4N_2-PEG

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.

FIG. 42F

CHO, BHK, 293 cells, Vero expressed DNase I.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1;
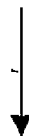j-m (independently selected) = 0-20;
v-y (independently selected) = 0.

FIG. 42G

$$\text{H}_2\text{N} \underset{\substack{\uparrow \\ \text{A} \\ 40}}{\rule{4cm}{0.4pt}} \underset{\substack{\uparrow \\ \text{A} \\ 128}}{\rule{4cm}{0.4pt}} \text{COOH}$$

a-d, i, n, p-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 42H

Insect cell expressed DNase I.
a-d, f, h, j-n, s, u, v-y' = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.

    1. GNT's 1,2,4,5, UDP-GlcNAc
    2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1; j-n = 0;
v-y (independently selected) = 1,
   when e-h (independently selected) is 1;
R = PEG.

FIG. 42I

Yeast expressed DNase I.
a-n = 0;
q-y (independently selected) = 0 to 1;
p = 1; R (branched or linear) = Man, oligomannose.

    1. Endoglycanase
    2. Galactosyltransferase, UDP-Gal
    3. CMP-SA-PEG, ST3Gal3

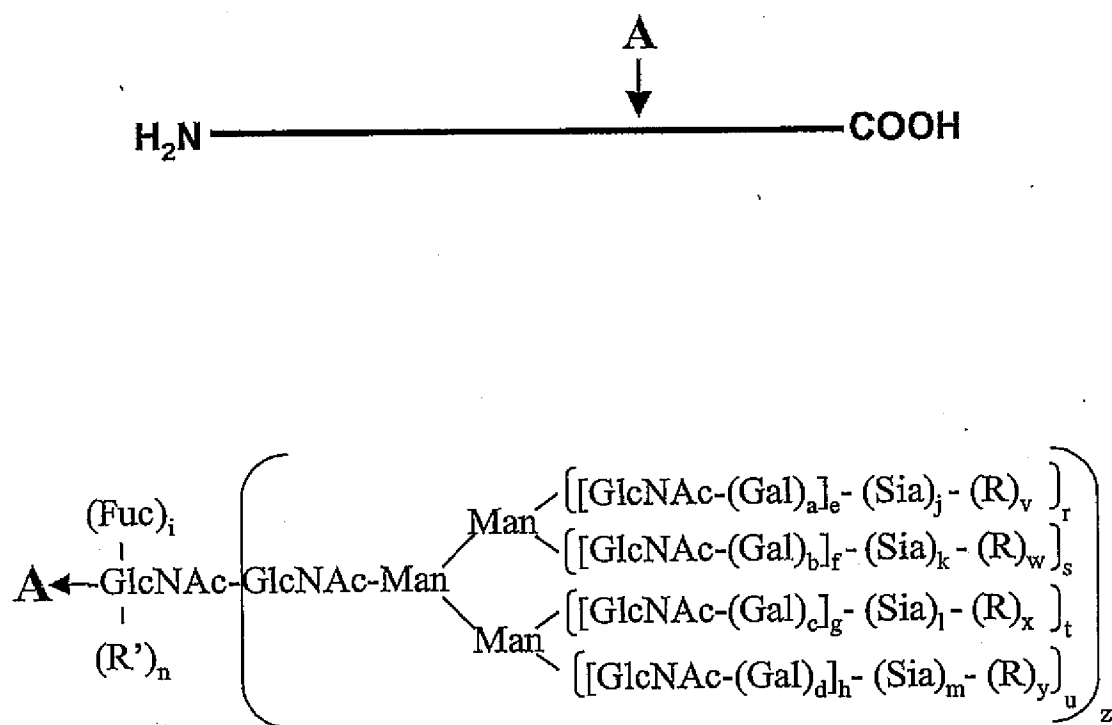a-n, p-y = 0; n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.

FIG. 42J

CHO, BHK, 293 cells, Vero expressed DNase I.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; n, v-y = 0.

1. CMP-SA-linker-SA-CMP, ST3Gal3
2. ST3Gal1, desialylated alpha-1-
   Proteinase inhibitor.
3. CMP-SA, ST3Gal3, ST3Gal1

a-m, q-u (independently selected) = 0 or 1;
p = 1; n = 0;
v-y (independently selected) = 0 or 1;
R = linker- alpha-1-Proteinase inhibitor.

FIG. 42K

$$\overset{\overset{\textbf{A}}{\downarrow}}{\text{H}_2\text{N}\text{------------------COOH}}$$

$$\begin{array}{c} (\text{Fuc})_i \\ | \\ \textbf{A} \leftarrow \text{GlcNAc-GlcNAc-Man} \\ | \\ (\text{R}')_n \end{array} \left(\begin{array}{c} \text{Man} \overset{\displaystyle \left[[\text{GlcNAc-(Gal)}_a]_e - (\text{Sia})_j - (\text{R})_v \right]_r}{\displaystyle \left[[\text{GlcNAc-(Gal)}_b]_f - (\text{Sia})_k - (\text{R})_w \right]_s} \\ \text{Man} \overset{\displaystyle \left[[\text{GlcNAc-(Gal)}_c]_g - (\text{Sia})_l - (\text{R})_x \right]_t}{\displaystyle \left[[\text{GlcNAc-(Gal)}_d]_h - (\text{Sia})_m - (\text{R})_y \right]_u} \end{array} \right)_z$$

a-d, i, r-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 4.
j-m (independently selected) = 0 or 1.
n, v-y = 0; z = 0 or 1;
R = modifying group, mannose, oligo-mannose;
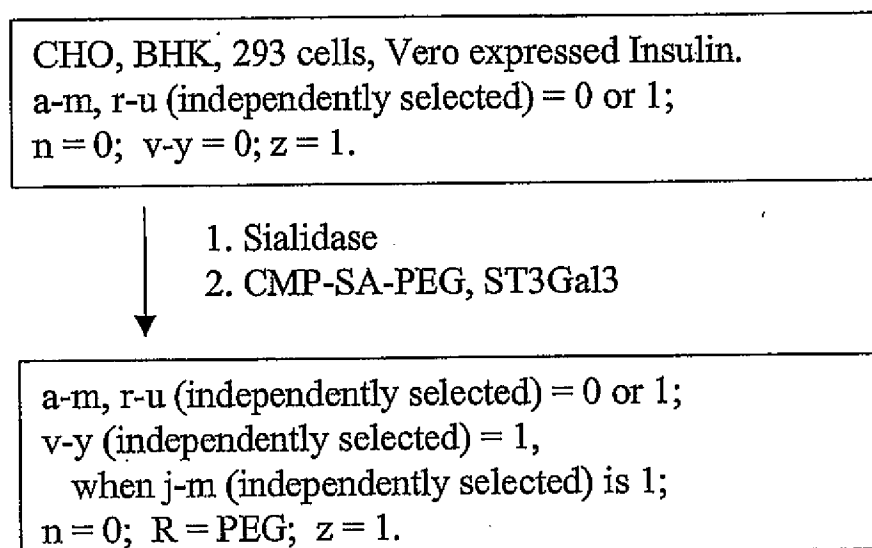R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 43A

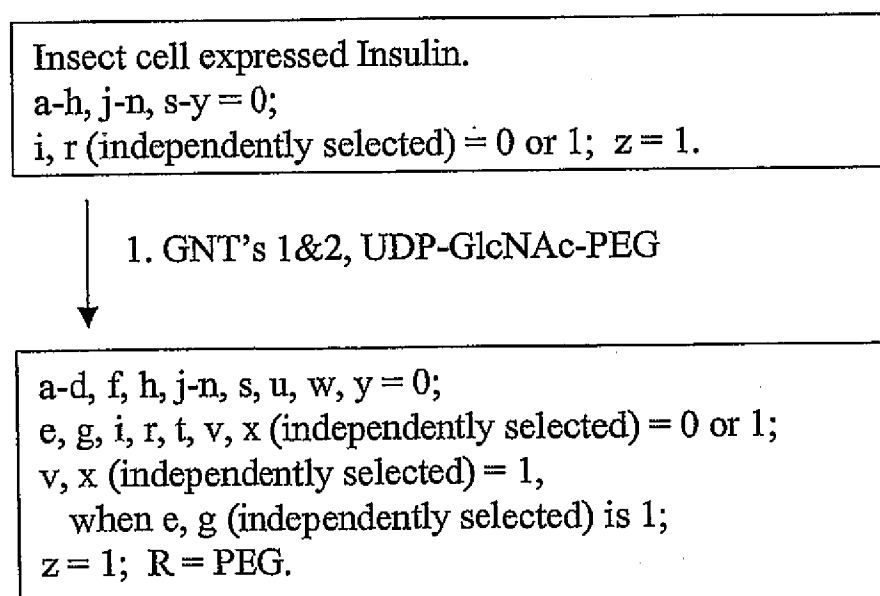CHO, BHK, 293 cells, Vero expressed Insulin.
a-m, r-u (independently selected) = 0 or 1;
n = 0; v-y = 0; z = 1.

    1. Sialidase
    2. CMP-SA-PEG, ST3Gal3

a-m, r-u (independently selected) = 0 or 1;
v-y (independently selected) = 1,
   when j-m (independently selected) is 1;
n = 0; R = PEG; z = 1.

FIG. 43B

Insect cell expressed Insulin.
a-h, j-n, s-y = 0;
i, r (independently selected) = 0 or 1; z = 1.

    1. GNT's 1&2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, v, x (independently selected) = 0 or 1;
v, x (independently selected) = 1,
   when e, g (independently selected) is 1;
z = 1; R = PEG.

FIG. 43C

Yeast expressed Insulin.
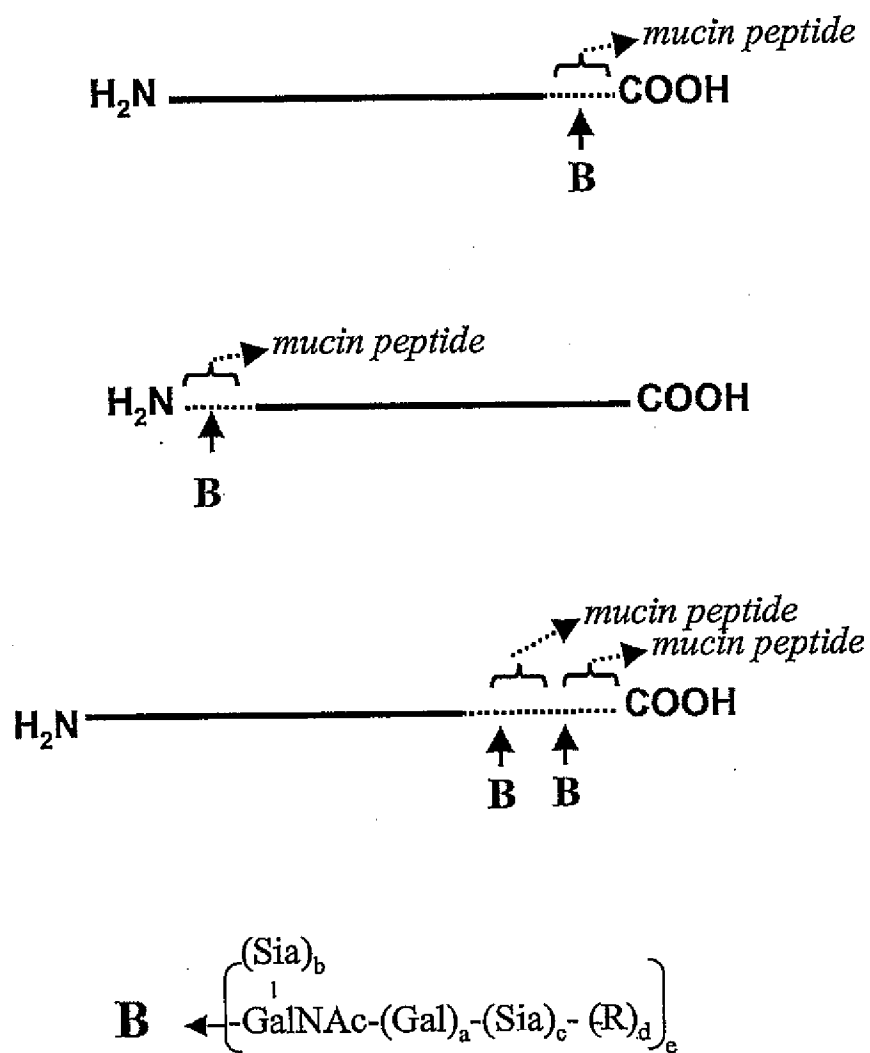a-n = 0; r-y (independently selected) = 0 to 1;
z = 1;
R (branched or linear) = Man, oligomannose or
    polysaccharide.

1. Endo-H
2. Galactosyltransferase, UDP-Gal-PEG

a-m, r-z = 0; n = 1; R' = -Gal-PEG.

FIG. 43D

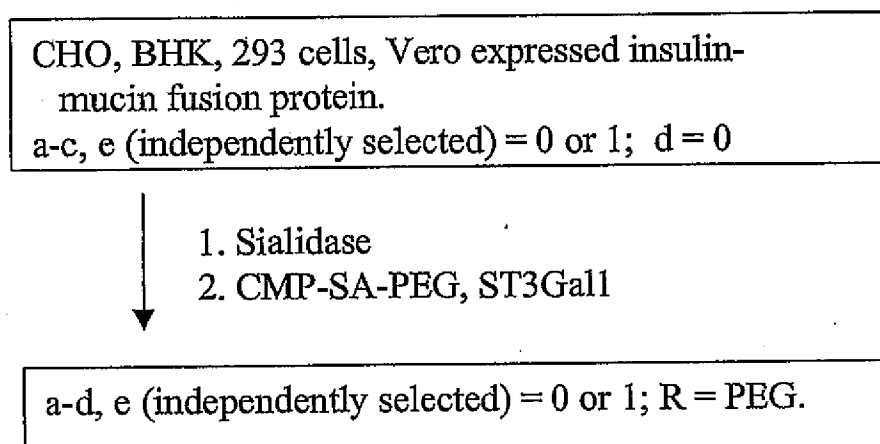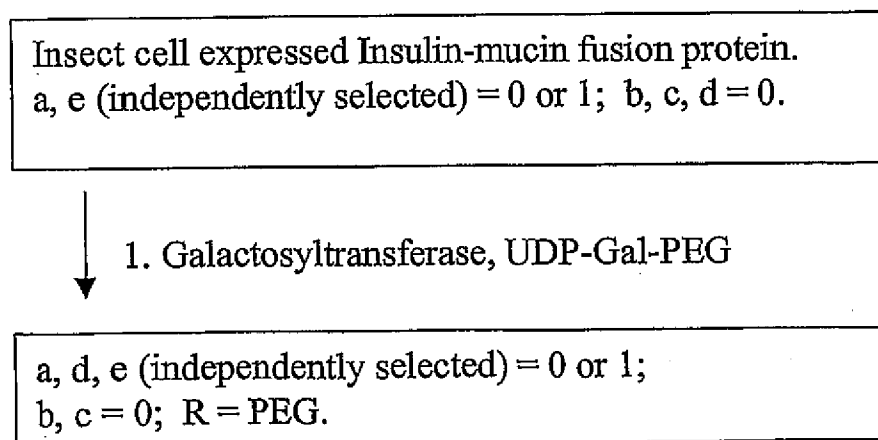a-c, e (independently selected) = 0 or 1;
d = 0; R = polymer

FIG. 43E

CHO, BHK, 293 cells, Vero expressed insulin-
  mucin fusion protein.
a-c, e (independently selected) = 0 or 1; d = 0

1. Sialidase
2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

FIG. 43F

Insect cell expressed Insulin-mucin fusion protein.
a, e (independently selected) = 0 or 1; b, c, d = 0.

1. Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1;
b, c = 0; R = PEG.

FIG. 43G

E. coli expressed Insulin-mucin fusion protein.
a-e = 0.

1. GalNAc Transferase, UDP-GalNAc
2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1;
a, b = 0; R = PEG.

FIG. 43H

195/345

FIG. 43I

E. coli expressed Insulin-mucin fusion protein.
a-e, n = 0.

1. GalNAc Transferase,
   UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1;
a-c, n = 0; R = PEG.

FIG. 43J

E. coli expressed Insulin-mucin fusion protein.
a-e, n = 0.

1. GalNAc Transferase,
   UDP-GalNAc-linker-SA-CMP
2. ST3Gal3, asialo-transferrin
3. CMP-SA, ST3Gal3

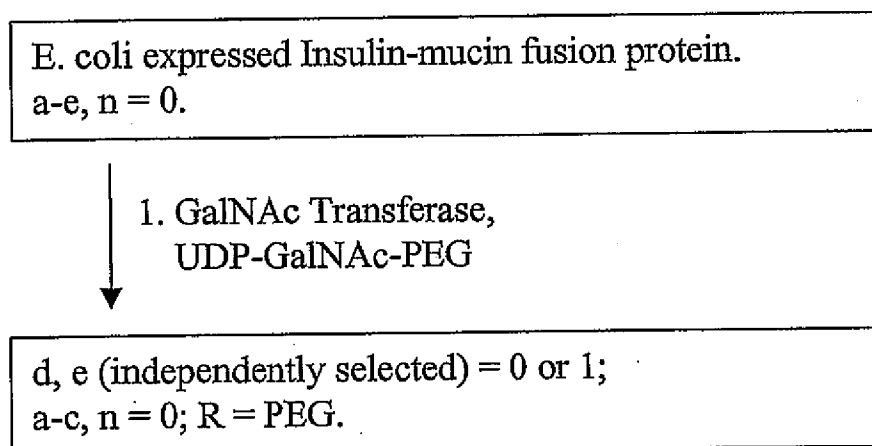d, e (independently selected) = 0 or 1;
a-c, n = 0; R = linker-transferrin.

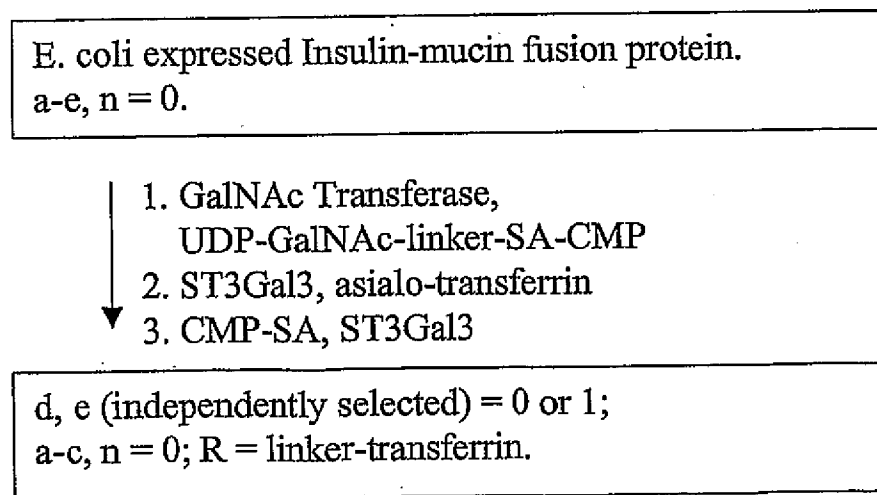## FIG. 43K

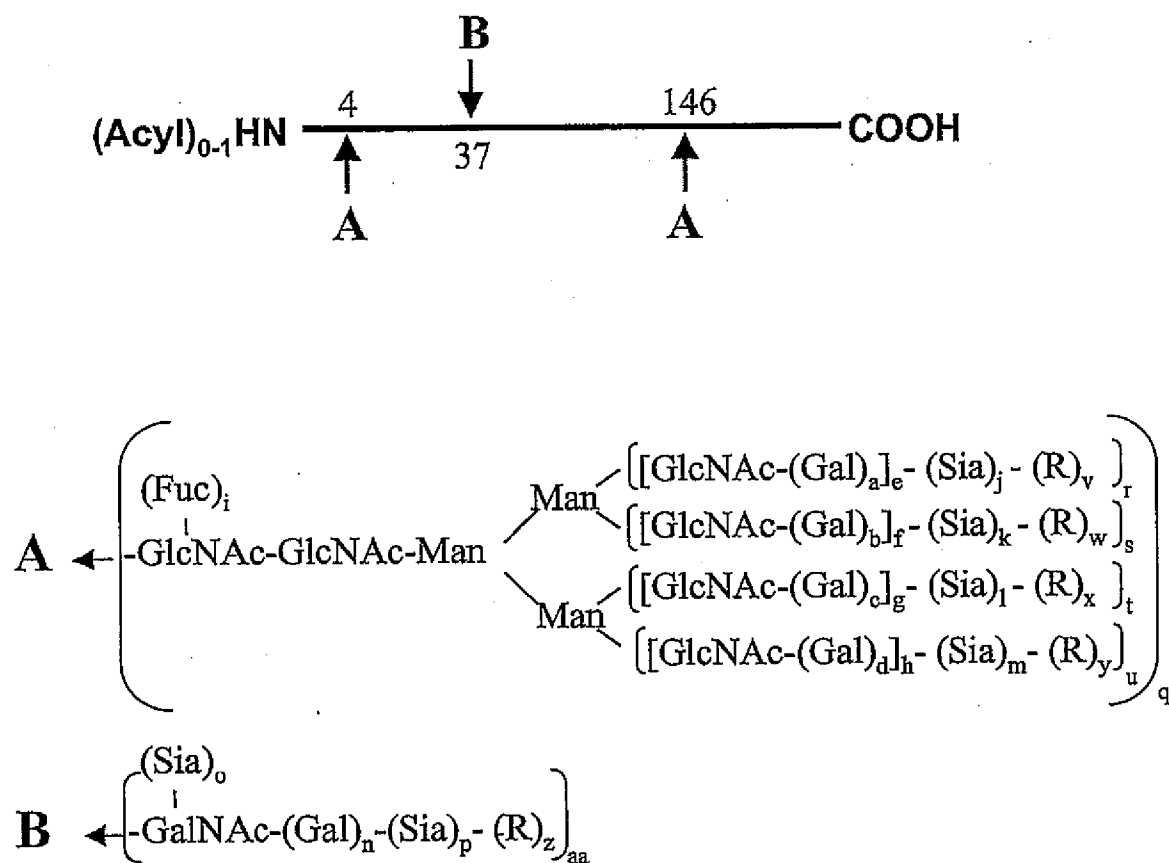E. coli expressed Insulin (N)—no mucin peptide.
a-e, n = 0.

1. NHS-CO-linker-SA-CMP
2. ST3Gal3, asialo-transferrin
3. CMP-SA, ST3Gal3

a-e = 0; n = 1;
R' = linker-transferrin.

FIG. 43L

$$(Acyl)_{0-1}HN \quad \overset{4}{\underset{37}{\underline{\quad\quad\quad\quad}}} \quad \overset{B}{\downarrow} \quad \overset{146}{\underline{\quad\quad\quad}} \quad COOH$$

$$A \leftarrow \left[\begin{array}{c} (Fuc)_i \\ | \\ -GlcNAc-GlcNAc-Man \end{array} \begin{array}{l} Man \nwarrow^{\displaystyle \left[[GlcNAc-(Gal)_a]_e - (Sia)_j - (R)_v \right]_r} _{\displaystyle \left[[GlcNAc-(Gal)_b]_f - (Sia)_k - (R)_w \right]_s} \\ Man \nwarrow^{\displaystyle \left[[GlcNAc-(Gal)_c]_g - (Sia)_l - (R)_x \right]_t} _{\displaystyle \left[[GlcNAc-(Gal)_d]_h - (Sia)_m - (R)_y \right]_u} \end{array} \right]_q$$

$$B \leftarrow \left[\begin{array}{c} (Sia)_o \\ | \\ -GalNAc-(Gal)_n-(Sia)_p- (R)_z \end{array} \right]_{aa}$$

a-d, i, n-u, aa (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0; R = polymer, glycoconjugate.

FIG. 44A

CHO, BHK, 293 cells, Vero expressed M-antigen.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z = 0.

1. Sialidase
2. CMP-SA-linker-lipid-A,
   ST3Gal3

a-d, i-m, q-u, aa (independently selected) = 0 or 1;
o, p, z = 0; n, e-h = 1;
v-y (independently selected) = 1,
   when j-m (independently selected) is 1;
R = linker-lipid-A.

FIG. 44B

CHO, BHK, 293 cells, Vero expressed M-antigen.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z = 0.

1. sialidase
2. CMP-SA-linker-tetanus toxin, ST3Gal1
3. CMP-SA, ST3Gal3

a-d, i-m, p-u, z, aa (independently selected) = 0 or 1;
o, v-y = 0; n, e-h = 1; R = tetanus toxin.

FIG. 44C

NSO expressed M-antigen.
a-d, i-n, o-u, aa (independently selected) = 0 or 1;
e-h = 1; v-z = 0;
Sia (independently selected) = Sia or Gal.

1. α-galactosidase
2. CMP-SA, ST3Gal3
2. CMP-SA-KLH, ST3Gal1

a-d, i-n, p-u, z, aa (independently selected) = 0 or 1;
e-h = 1; o, v-y = 0;
z = 1, when p = 1;
R = KLH.

FIG. 44D

Yeast expressed M-antigen.
a-p, z = 0; q-y, aa (independently selected) = 0 to 1;
R (branched or linear) = Man, oligomannose;
GalNAc = Man.

1. α1,2-mannosidase
2. GNT 1,
   UDP-GlcNAc-linker-diphtheria toxin.

e, q, l, m, r, t, u, v, aa (independently selected) =0 or 1;
a-d, f-h, j, k, n-p, s, w-z = 0;
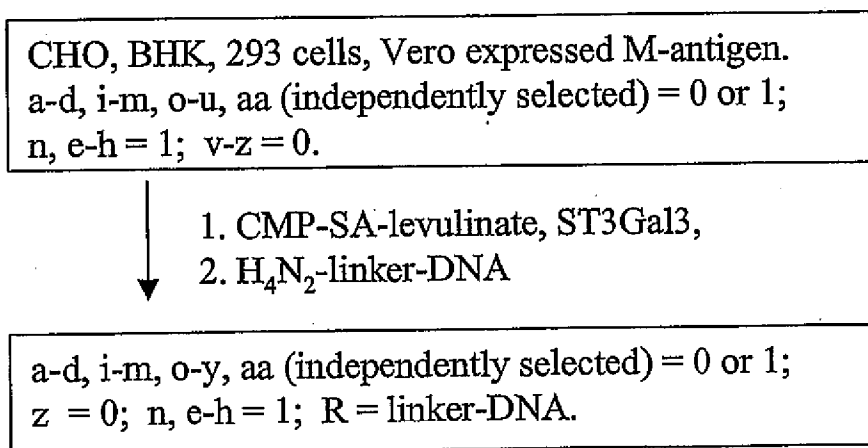Sia = Man; R = linker-diphtheria toxin.

FIG. 44E

CHO, BHK, 293 cells, Vero expressed M-antigen.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z = 0.

↓ 1. CMP-SA-levulinate, ST3Gal3,
   2. H_4N_2-linker-DNA

a-d, i-m, o-y, aa (independently selected) = 0 or 1;
z = 0; n, e-h = 1; R = linker-DNA.

FIG. 44F

CHO, BHK, 293 cells, Vero expressed M-antigen.
a-d, i-n, o-u, aa (independently selected) = 0 or 1;
e-h = 1; v-z = 0.

↓ 1. CMP-SA, poly-α2,8-ST

a-d, i, n-u, aa (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-100;
v-z (independently selected) = 0.

FIG. 44G

$(Acyl)_{0-1}HN$ —|4— B↓ —146— COOH
37
A↑ A↑

$$A \leftarrow \left\{ \begin{array}{c} (Fuc)_i \\ | \\ \text{-GlcNAc-GlcNAc-Man} \\ | \\ (R')_{cc} \end{array} \left\{ \begin{array}{c} Man \left< \begin{array}{c} \left[[GlcNAc\text{-}(Gal)_a]_e\text{-}(Sia)_j\text{-}(R)_v \right]_r \\ \left[[GlcNAc\text{-}(Gal)_b]_f\text{-}(Sia)_k\text{-}(R)_w \right]_s \end{array} \right. \\ Man \left< \begin{array}{c} \left[[GlcNAc\text{-}(Gal)_c]_g\text{-}(Sia)_l\text{-}(R)_x \right]_t \\ \left[[GlcNAc\text{-}(Gal)_d]_h\text{-}(Sia)_m\text{-}(R)_y \right]_u \end{array} \right. \end{array} \right._q \right\}_{bb}$$

$$B \leftarrow \left\{ \begin{array}{c} (Sia)_o \\ | \\ \text{-GalNAc-}(Gal)_n\text{-}(Sia)_p\text{-}(R)_z \end{array} \right\}_{aa}$$
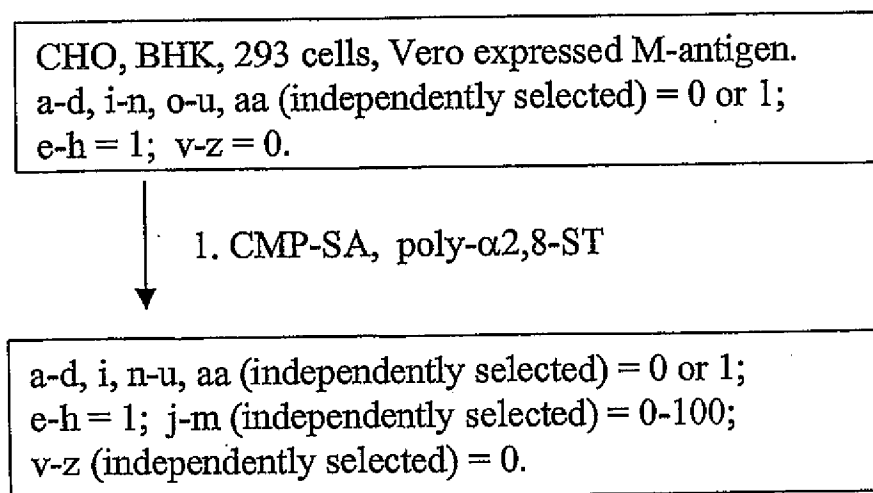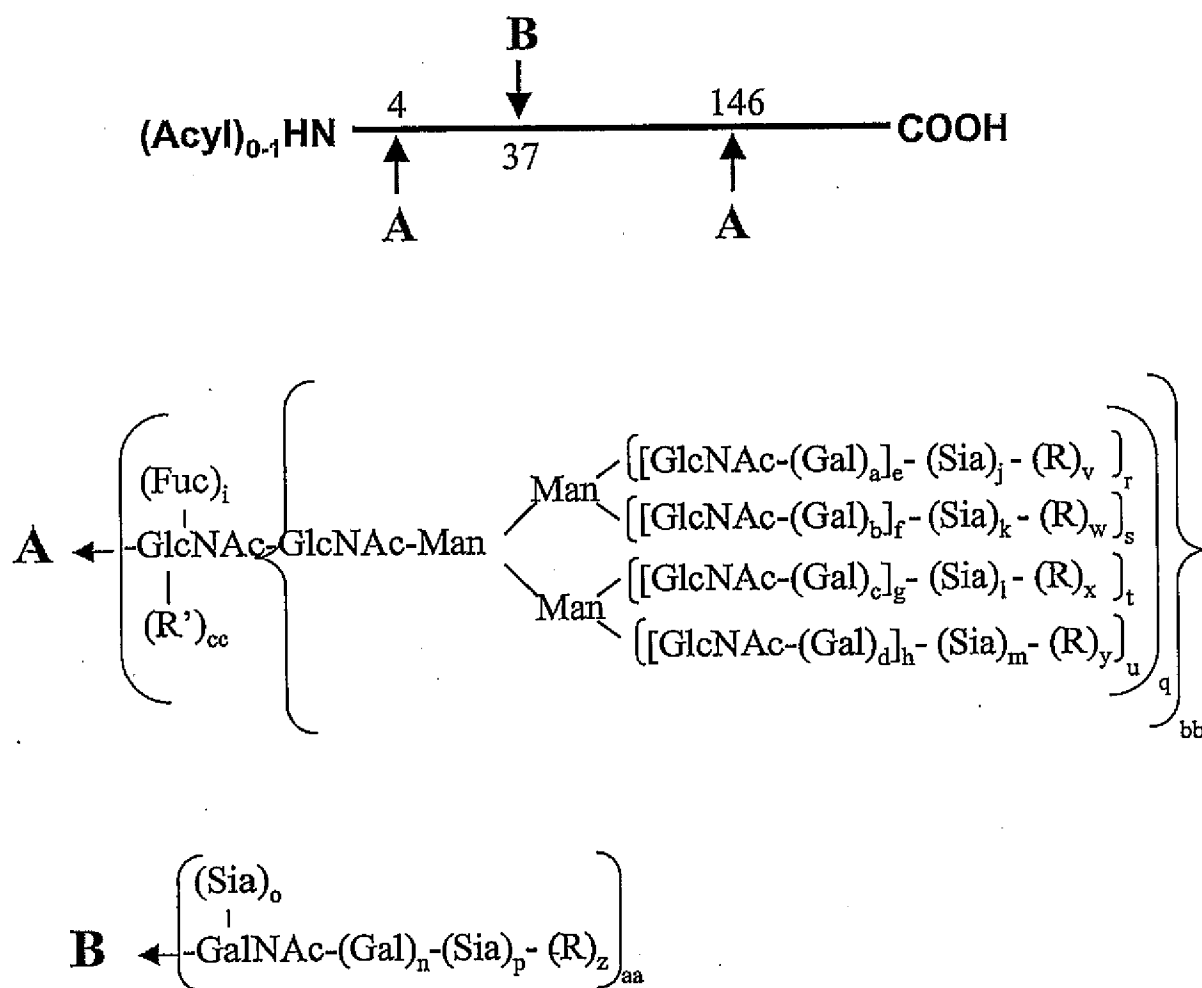
a-d, i, n, q-u, aa, bb, (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-p (independently selected) = 0 to 100.
Cc, v-y = 0;
R = modifying group, mannose, oligo-mannose.
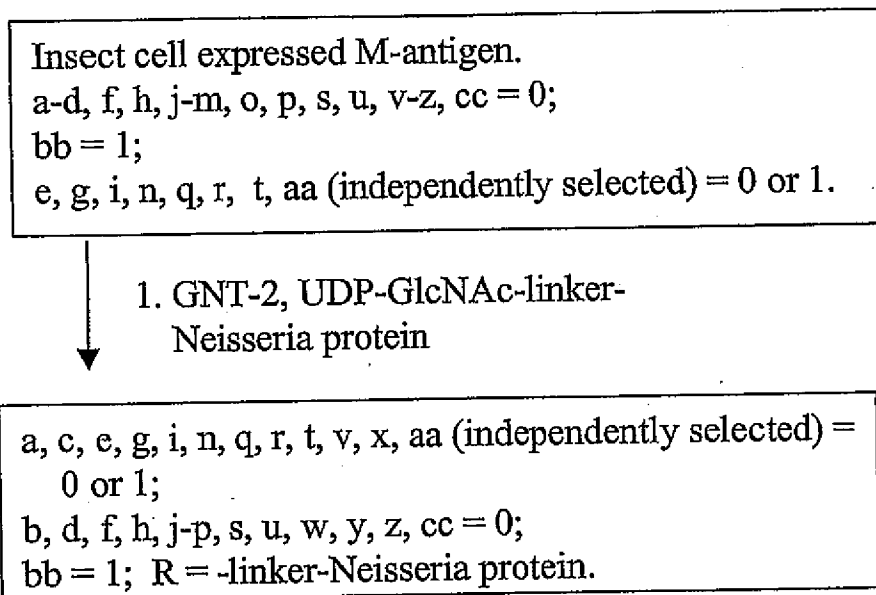R'= H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 44H

Insect cell expressed M-antigen.
a-d, f, h, j-m, o, p, s, u, v-z, cc = 0;
bb = 1;
e, g, i, n, q, r, t, aa (independently selected) = 0 or 1.

1. GNT-2, UDP-GlcNAc-linker-
   Neisseria protein

a, c, e, g, i, n, q, r, t, v, x, aa (independently selected) =
   0 or 1;
b, d, f, h, j-p, s, u, w, y, z, cc = 0;
bb = 1; R = -linker-Neisseria protein.

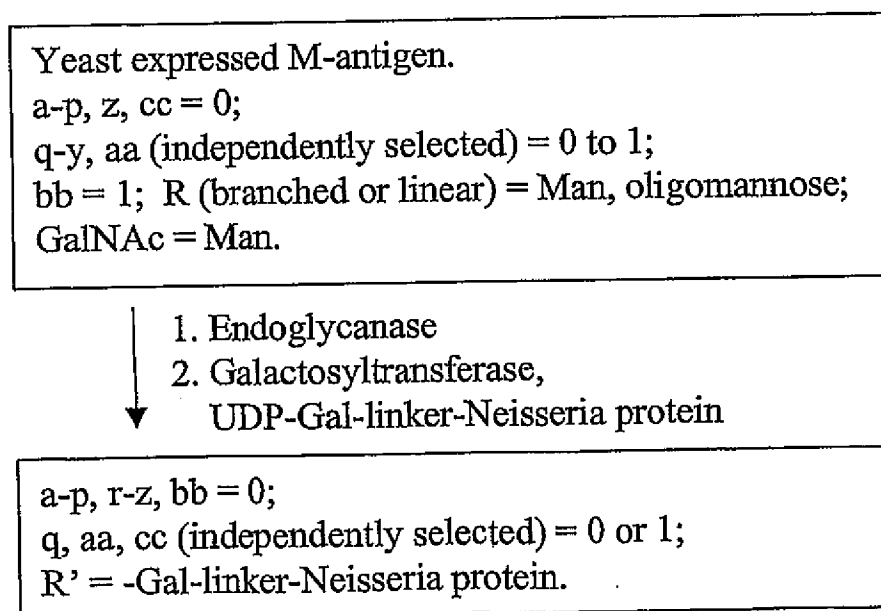# FIG. 44I

Yeast expressed M-antigen.
a-p, z, cc = 0;
q-y, aa (independently selected) = 0 to 1;
bb = 1; R (branched or linear) = Man, oligomannose;
GalNAc = Man.

1. Endoglycanase
2. Galactosyltransferase,
   UDP-Gal-linker-Neisseria protein

a-p, r-z, bb = 0;
q, aa, cc (independently selected) = 0 or 1;
R' = -Gal-linker-Neisseria protein.

FIG. 44J

Yeast expressed M-antigen.
a-p, z, cc = 0;
q-y, aa (independently selected) = 0 to 1; bb = 1;
R (branched or linear) = Man, oligomannose;
GalNAc = Man.

1. mannosidases
2. GNT 1 & 2, UDP-GlcNAc
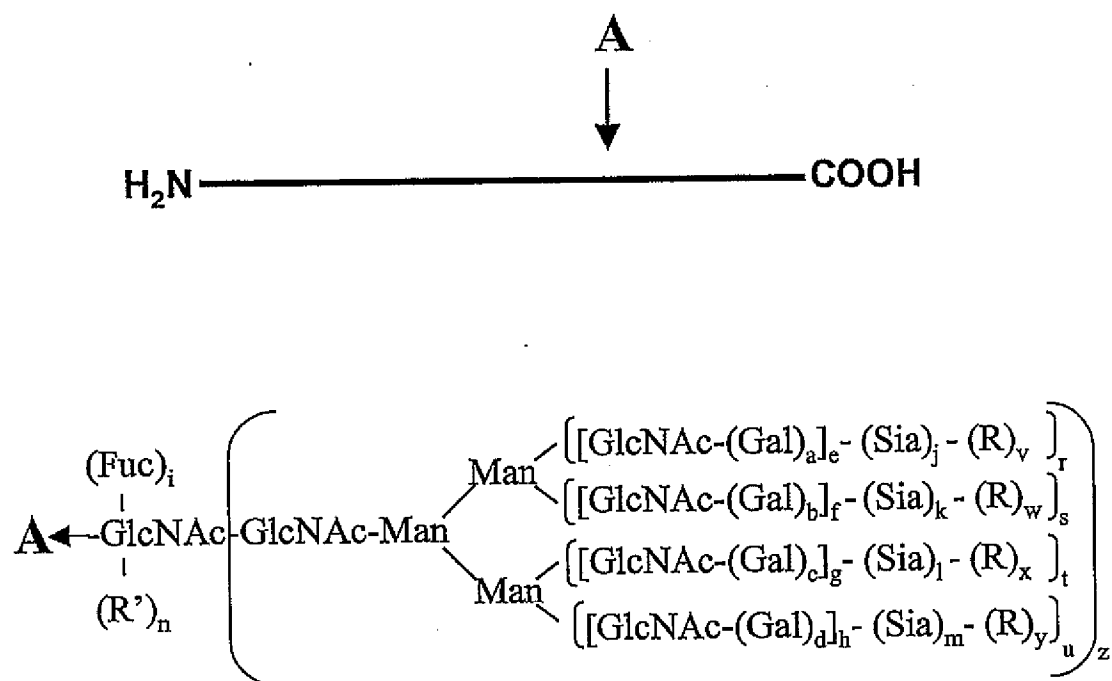3. UDP-Gal, Galactosyltransferase,
4. CMP-SA, sialyltransferase

a, c, e, g, j, l, q, r, t, aa (independently selected) = 0 or 1;
b, d, f, h, k, m-p, s, u-z, cc = 0; bb = 1.

FIG. 44K

$$\text{A} \downarrow$$

$$\text{H}_2\text{N} \overline{\hspace{4cm}} \text{COOH}$$

$$\begin{array}{c}(\text{Fuc})_i \\ | \\ \text{A} \blacktriangleleft \text{GlcNAc} \text{—GlcNAc-Man} \\ | \\ (\text{R'})_n \end{array} \left(\begin{array}{c} \text{Man} \begin{array}{c} \left[[\text{GlcNAc-(Gal)}_a]_e \text{-} (\text{Sia})_j \text{-} (\text{R})_v \right]_r \\ \left[[\text{GlcNAc-(Gal)}_b]_f \text{-} (\text{Sia})_k \text{-} (\text{R})_w \right]_s \end{array} \\ \text{Man} \begin{array}{c} \left[[\text{GlcNAc-(Gal)}_c]_g \text{-} (\text{Sia})_l \text{-} (\text{R})_x \right]_t \\ \left[[\text{GlcNAc-(Gal)}_d]_h \text{-} (\text{Sia})_m \text{-} (\text{R})_y \right]_u \end{array} \end{array} \right)_z$$

a-d, i, r-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 4.
j-m (independently selected) = 0 or 1.
n, v-y = 0; z = 0 or 1;
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 45A

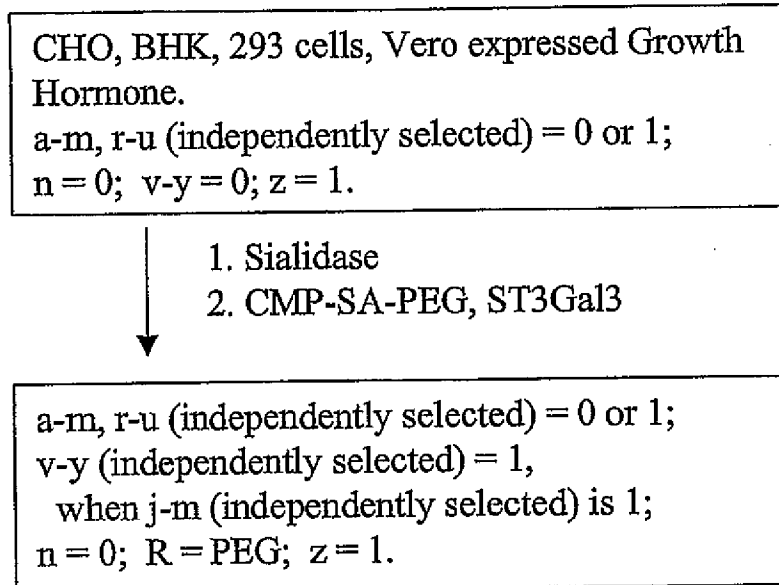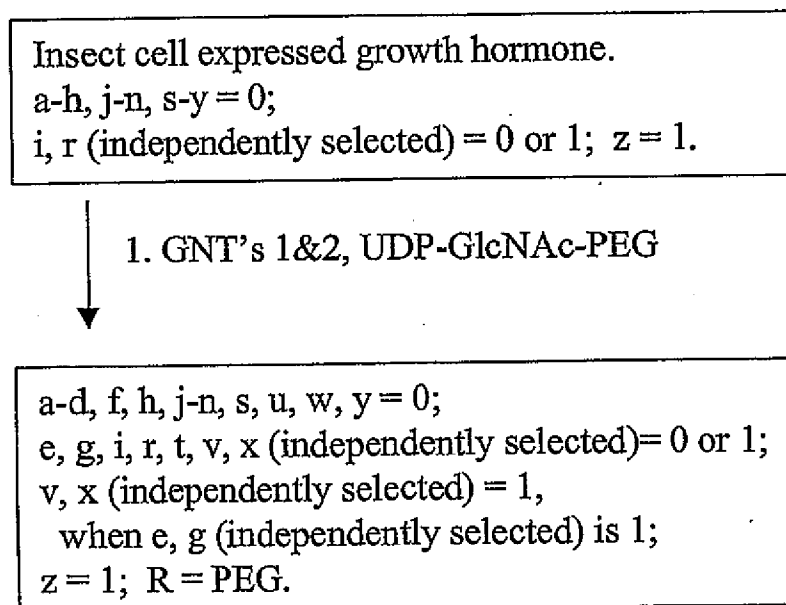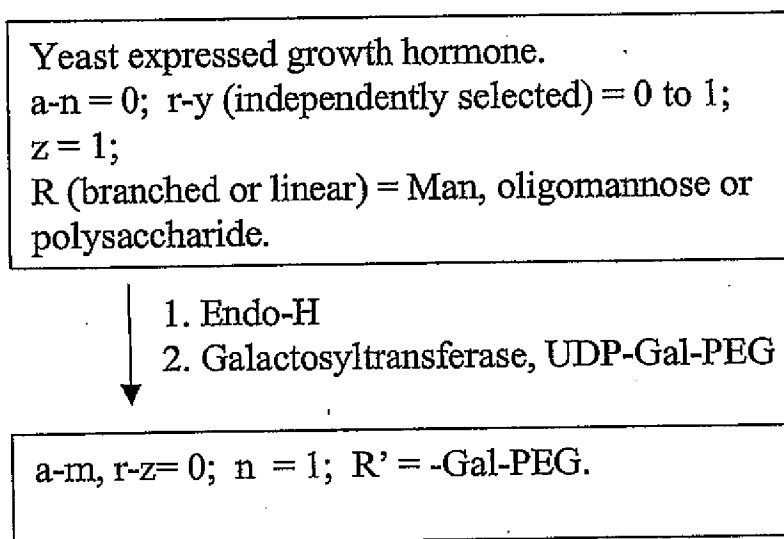CHO, BHK, 293 cells, Vero expressed Growth
Hormone.
a-m, r-u (independently selected) = 0 or 1;
n = 0; v-y = 0; z = 1.

1. Sialidase
2. CMP-SA-PEG, ST3Gal3

a-m, r-u (independently selected) = 0 or 1;
v-y (independently selected) = 1,
   when j-m (independently selected) is 1;
n = 0; R = PEG; z = 1.

FIG. 45B

Insect cell expressed growth hormone.
a-h, j-n, s-y = 0;
i, r (independently selected) = 0 or 1; z = 1.

1. GNT's 1&2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, v, x (independently selected)= 0 or 1;
v, x (independently selected) = 1,
   when e, g (independently selected) is 1;
z = 1; R = PEG.

FIG. 45C

Yeast expressed growth hormone.
a-n = 0; r-y (independently selected) = 0 to 1;
z = 1;
R (branched or linear) = Man, oligomannose or polysaccharide.

1. Endo-H
2. Galactosyltransferase, UDP-Gal-PEG

a-m, r-z = 0; n = 1; R' = -Gal-PEG.

FIG. 45D

a-c, e (independently selected) = 0 or 1;
d = 0;
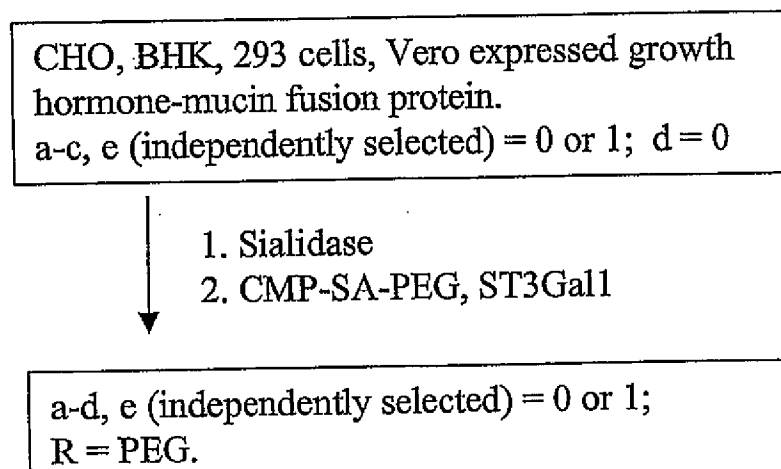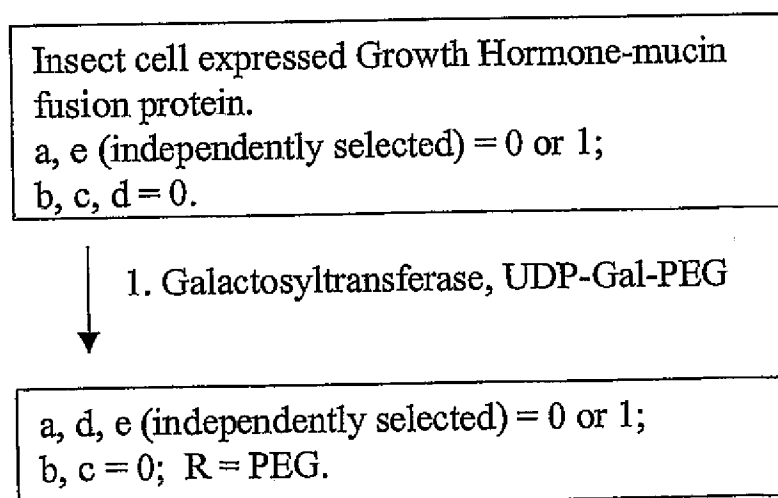R = modifying group, mannose, oligo-
mannose.

FIG. 45E

CHO, BHK, 293 cells, Vero expressed growth
hormone-mucin fusion protein.
a-c, e (independently selected) = 0 or 1; d = 0

   1. Sialidase
   2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1;
R = PEG.

FIG. 45F

Insect cell expressed Growth Hormone-mucin
fusion protein.
a, e (independently selected) = 0 or 1;
b, c, d = 0.

   1. Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1;
b, c = 0; R = PEG.

FIG. 45G

E. coli expressed growth hormone-mucin
    fusion protein.
a-e = 0.

1. GalNAc Transferase, UDP-GalNAc
2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1;
a, b = 0; R = PEG.

FIG. 45H

E. coli expressed growth hormone-mucin fusion
protein.
a-e, n = 0.

1. GalNAc Transferase,
    UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1;
a-c, n = 0; R = PEG.

FIG. 45I

E. coli expressed growth hormone-mucin fusion
protein.
a-e, n = 0.

    1. GalNAc Transferase,
       UDP-GalNAc-linker-SA-CMP
    2. ST3Gal3, asialo-transferrin
    3. CMP-SA, ST3Gal3

d, e (independently selected) = 0 or 1;
a-c, n = 0; R = linker-transferrin.

FIG. 45J

E. coli expressed growth hormone
  (N)—no mucin peptide.
a-e, n = 0.

    1. NHS-CO-linker-SA-CMP
    2. ST3Gal3, asialo-transferrin
    3. CMP-SA, ST3Gal3

a-e = 0; n = 1; R' = linker-transferrin.

FIG. 45K

a-d, i-m, q-u, w, z, nn, ww, zz (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

n, v-y = 0;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 46A

CHO, BHK, 293 cells, Vero or transgenic animals
    expressed TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1;
n = 1; v-z = 0.

1. CMP-SA, ST3Gal1
2. galactosyltransferase, UPD-Gal
3. CMP-SA-PEG, ST3Gal3

a-m, o-u, v-y, aa (independently selected) = 0 or 1;
n = 1; z = 0; R = PEG.

FIG. 46B

CHO, BHK, 293 cells, Vero expressed
    TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1;
n = 1; v-z = 0.

1. sialidase
2. CMP-SA-PEG, ST3Gal1

a-i, p-u, z, aa (independently selected) = 0 or 1;
n = 1; o, j-m, v-y = 0; R = PEG.

FIG. 46C

CHO, BHK, 293 cells, Vero expressed
  TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1;
n = 1; v-z = 0.

         1. galactosyltransferase, UPD-Gal-PEG

a-m, o-u, v-y, aa (independently selected) = 0 or 1;
n = 1; z = 0; R = PEG.

FIG. 46D

CHO, BHK, 293 cells, Vero or transgenic animals
  expressed TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1;
n = 1; v-z = 0.

      1. CMP-SA, ST3Gal1
      2. galactosyltransferase, UPD-Gal-PEG

a-m, o-u, v-y, aa (independently selected) = 0 or 1;
n = 1; z = 0; R = PEG.

FIG. 46E

CHO, BHK, 293 cells, Vero or transgenic animals
  expressed TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1;
n = 1; v-z = 0.

1. CMP-SA-levulinate, ST3Gal1
2. H_4N_2-PEG

a-m, o-u, v-y, aa (independently selected) = 0 or 1;
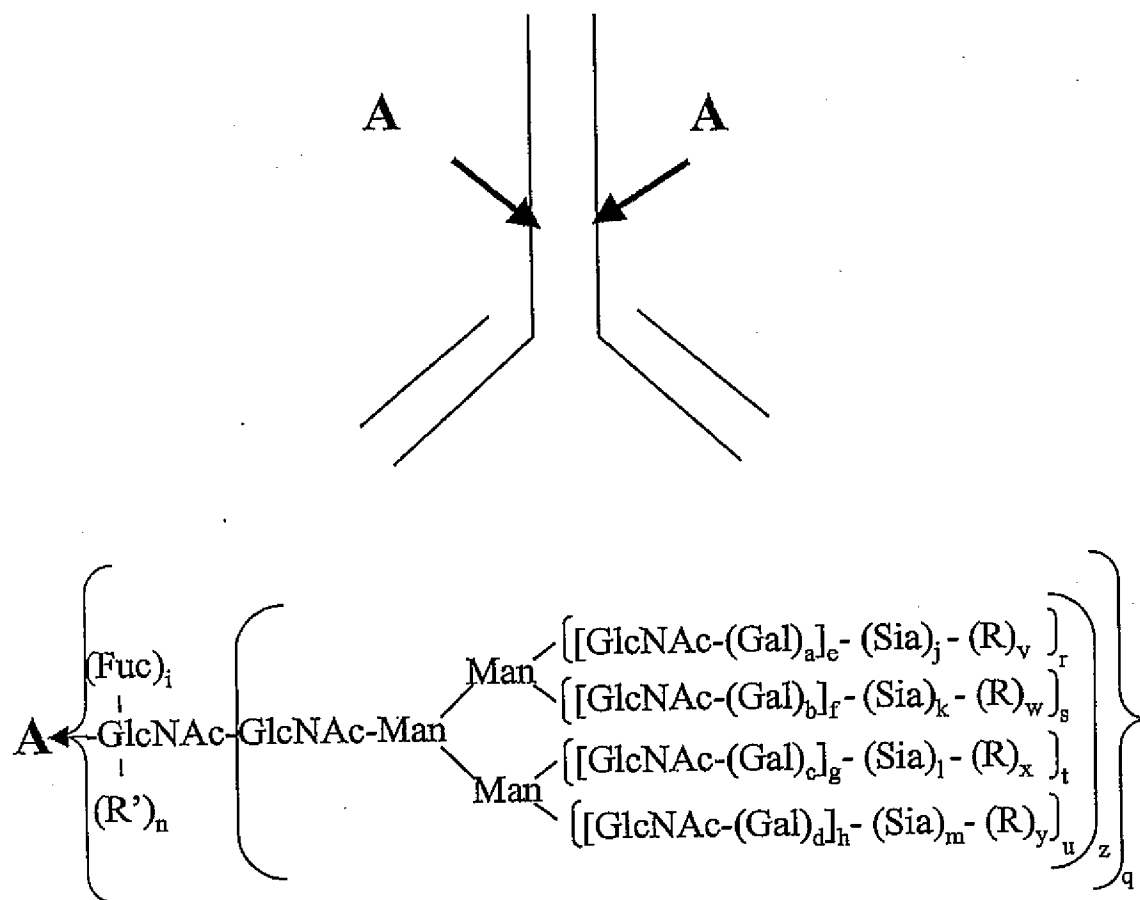n = 1; z = 0; R = PEG.

FIG. 46F

CHO, BHK, 293 cells, Vero expressed
  TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1;
n = 1; v-z = 0.

1. CMP-SA-PEG, α2,8-ST

a-i, o, q-u, v-z, aa (independently selected) = 0 or 1;
  n = 1; j-m, p (independently selected) = 0 to 2;
v-z (independently selected) = 1,
  when j-m, p (independently selected) is 2;
R = PEG.

FIG. 46G

a-d, i, l, q-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 4.
j-k (independently selected) = 0 or 1.
M = 0 to 20.
n, v-y = 0; z = 0 or 1;
R = polymer, toxin, radioisotope-complex, drug, mannose,
oligo-mannose.
R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 47A

CHO, BHK, 293 cells, Vero expressed Herceptin.
a, c, i (independently selected) = 0 or 1;
e, g, r, t = 1; b, d, f, h, j-m, n, s, u-y = 0;
q, z = 1.

       1. galactosyltransferase, UPD-Gal
       2. CMP-SA-toxin, ST3Gal3

a, c, i, j, 1 (independently selected) = 0 or 1;
e, g, r, t = 1; R = toxin;
 f, h, k, m, n, s, u-y = 0; q, z = 1;
v-y (independently selected) = 51,
   when j, 1 (independently selected) is 1.

FIG. 47B

CHO, BHK, 293 cells, Vero or fungal expressed
Herceptin.
a, c, i (independently selected) = 0 or 1;
e, g, r, t = 1; b, d, f, h, j-m, n, s, u-y = 0;
q, z = 1.

       1. galactosyltransferase,
         UPD-Gal-Toxin

a, c, i (independently selected) = 0 or 1;
e, g, r, t = 1; f, h, j-m, n, s, u-y = 0;
q, z = 1; v-y (independently selected) = 1,
   when a, c (independently selected) is 1;
R = toxin.

FIG. 47C

Fungi expressed Herceptin.
e, g, i, r, t (independently selected) = 0 or 1;
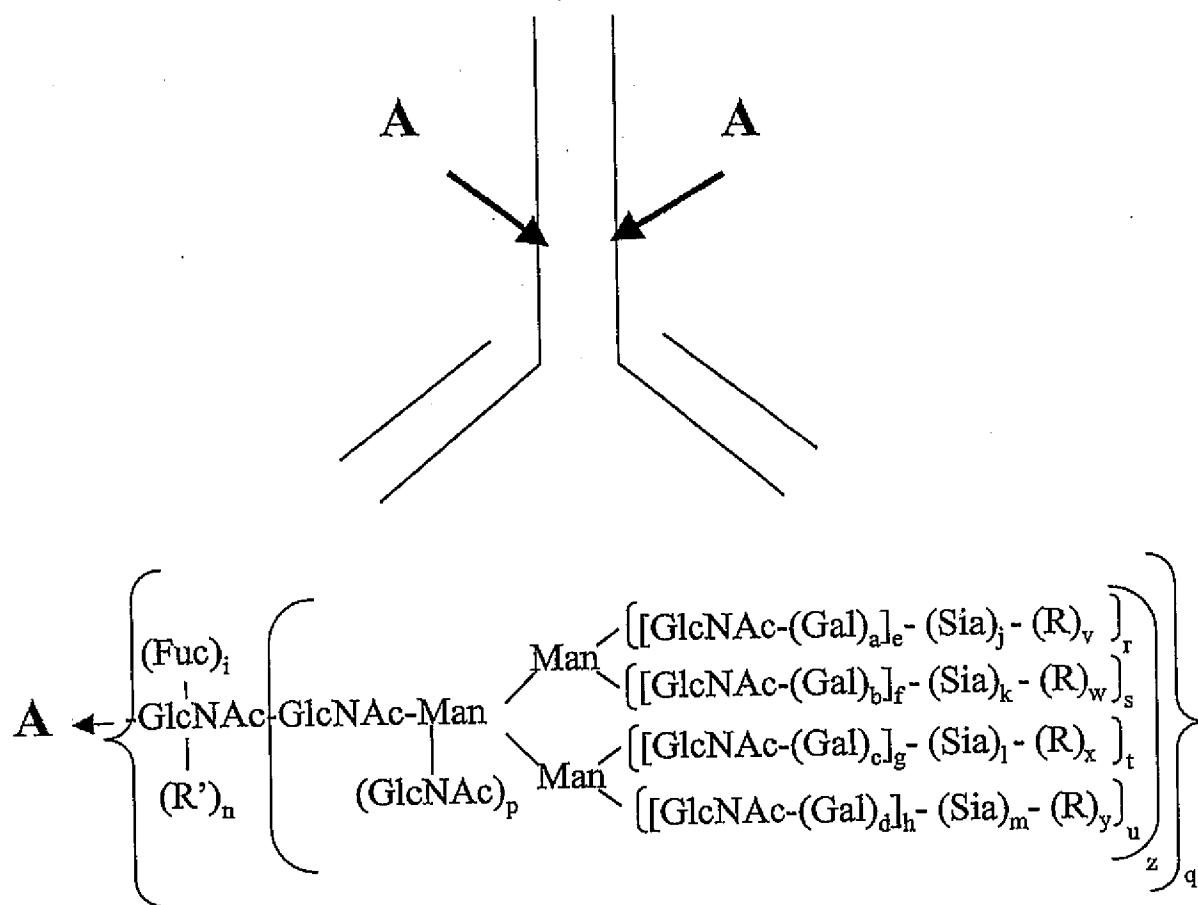a-d, f, h, j-m, n, s, u-y = 0; q, z = 1.

1. Endo-H
2. Galactosyltransferase, UDP-Gal
3.. CMP-SA-radioisotope complex, ST3Gal3

a-m, r-z= 0; q, n = 1;
R' = -Gal-Sia-radioisotope complex.

FIG. 47D

a-d, i, p-u, (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 or 1.

n, v-y = 0; z = 0 or 1;

R = polymer, toxin, radioisotope-complex, drug, mannose, oligo-mannose.

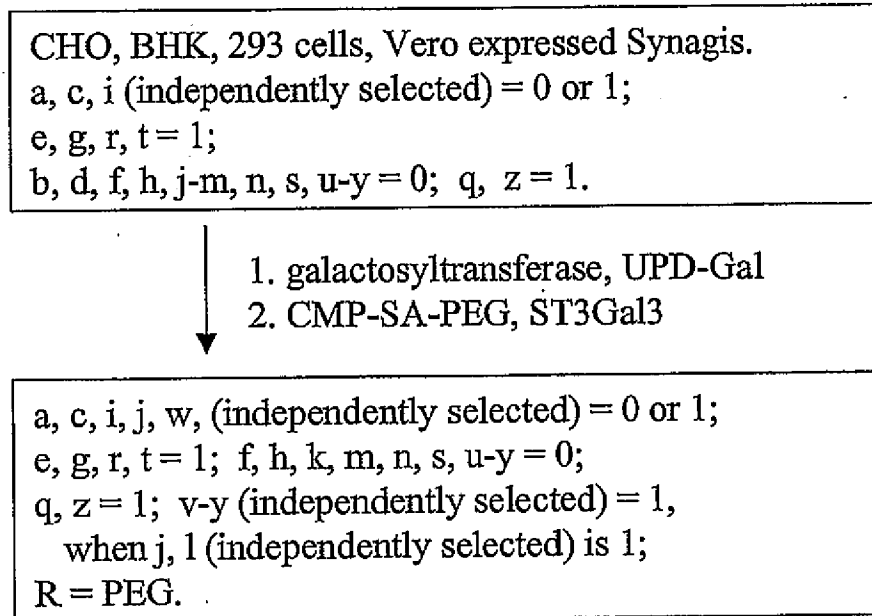R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 48A

CHO, BHK, 293 cells, Vero expressed Synagis.
a, c, i (independently selected) = 0 or 1;
$e, g, r, t = 1$;
$b, d, f, h, j\text{-}m, n, s, u\text{-}y = 0$; $q, z = 1$.

          ↓   1. galactosyltransferase, UPD-Gal
                2. CMP-SA-PEG, ST3Gal3

a, c, i, j, w, (independently selected) = 0 or 1;
$e, g, r, t = 1$; $f, h, k, m, n, s, u\text{-}y = 0$;
$q, z = 1$; $v\text{-}y$ (independently selected) = 1,
    when $j, 1$ (independently selected) is 1;
$R = PEG$.

FIG. 48B

CHO, BHK, 293 cells, Vero or fungal expressed
Synagis.
a, c, i (independently selected) = 0 or 1;
$e, g, r, t = 1$; $b, d, f, h, j\text{-}m, n, s, u\text{-}y = 0$;
$q, z = 1$.

          ↓   1. galactosyltransferase,
                UPD-Gal-PEG

a, c, i, w (independently selected) = 0 or 1;
$e, g, r, t = 1$; $f, h, j\text{-}m, n, s, u\text{-}y = 0$;
$q, z = 1$; $v\text{-}y$ (independently selected) = 1,
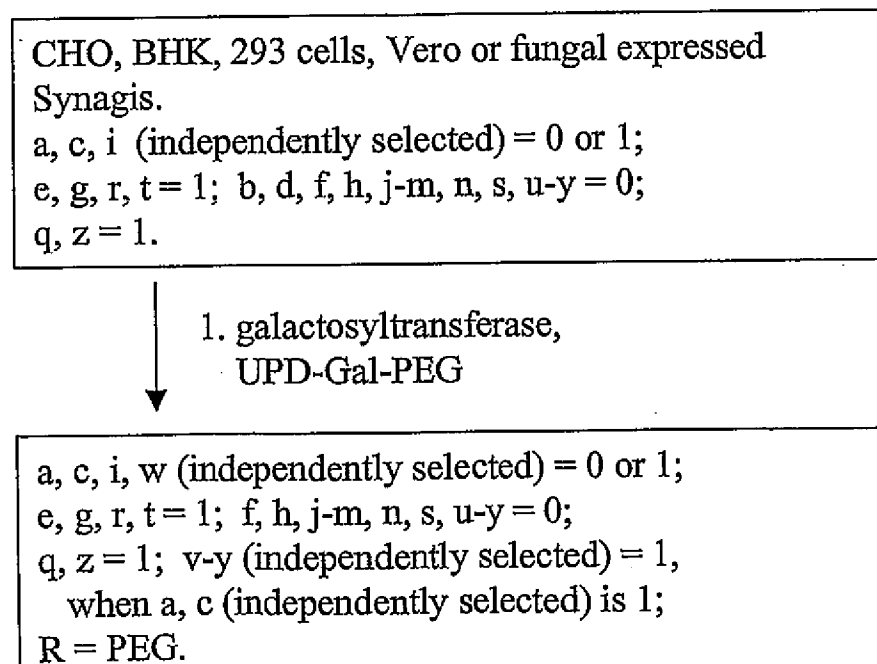   when a, c (independently selected) is 1;
$R = PEG$.

FIG. 48C

Fungi expressed Synagis.
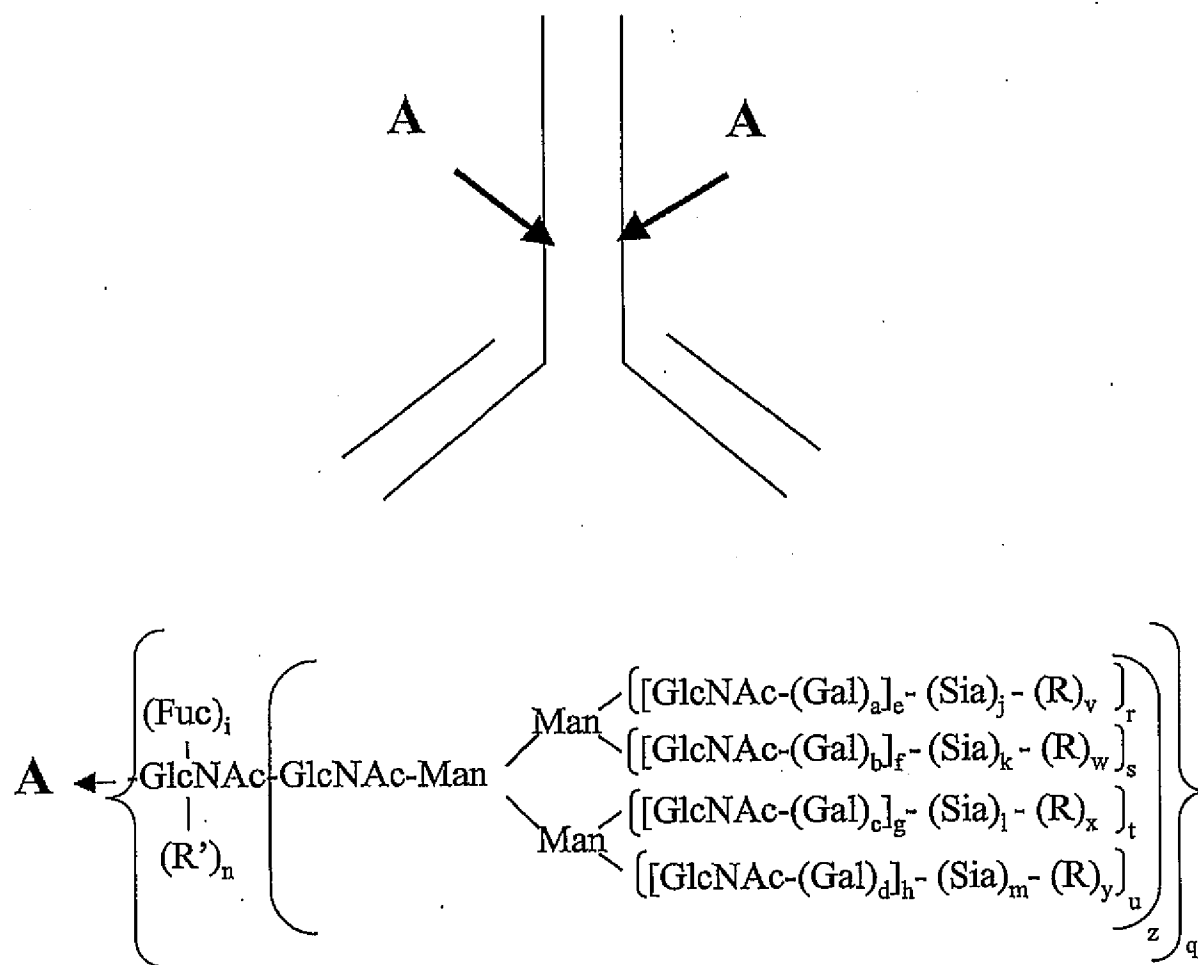e, g, i, r, t (independently selected) = 0 or 1;
a-d, f, h, j-m, n, s, u-y = 0; q, z = 1.

1. Endo-H
2. Galactosyltransferase, UDP-Gal
3.. CMP-SA-PEG, ST3Gal3

a-m, r-z= 0; q, n = 1; R' = -Gal-Sia-PEG.

FIG. 48D

a-d, i, q-u, w (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 20.

n, v-y = 0; z = 0 or 1;

R = polymer, toxin, radioisotope-complex, drug, mannose, oligo-mannose.

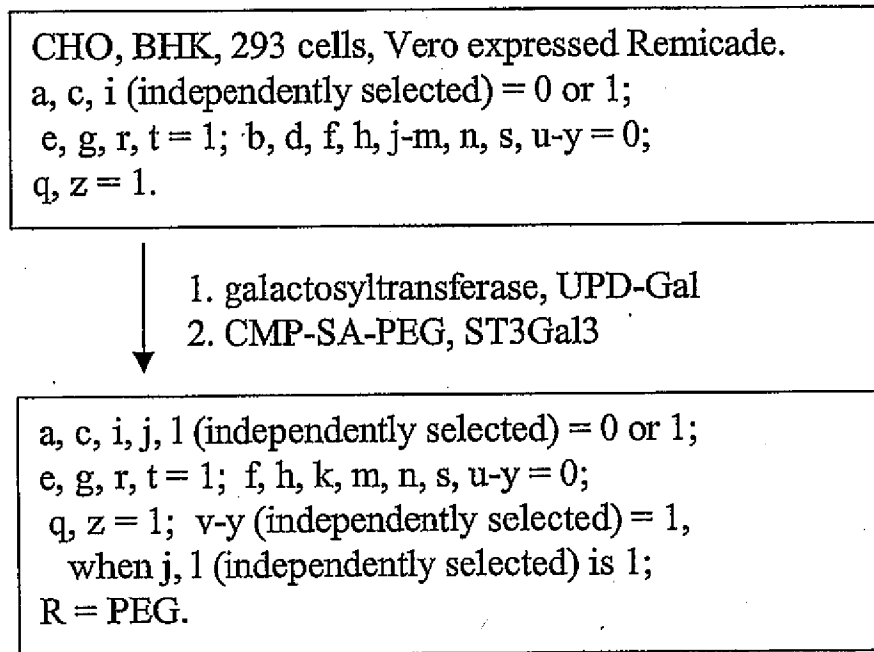R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 49A

CHO, BHK, 293 cells, Vero expressed Remicade.
a, c, i (independently selected) = 0 or 1;
e, g, r, t = 1; b, d, f, h, j-m, n, s, u-y = 0;
q, z = 1.

1. galactosyltransferase, UPD-Gal
2. CMP-SA-PEG, ST3Gal3

a, c, i, j, 1 (independently selected) = 0 or 1;
e, g, r, t = 1; f, h, k, m, n, s, u-y = 0;
q, z = 1; v-y (independently selected) = 1,
    when j, 1 (independently selected) is 1;
R = PEG.

FIG. 49B

CHO, BHK, 293 cells, Vero or fungal expressed
Remicade.
a, c, i (independently selected) = 0 or 1;
e, g, r, t = 1; b, d, f, h, j-m, n, s, u-y = 0;
q, z = 1.

1. galactosyltransferase,
    UPD-Gal-PEG

a, c, i (independently selected) = 0 or 1;
e, g, r, t = 1; f, h, j-m, n, s, u-y = 0;
q, z = 1; v-y (independently selected) = 1,
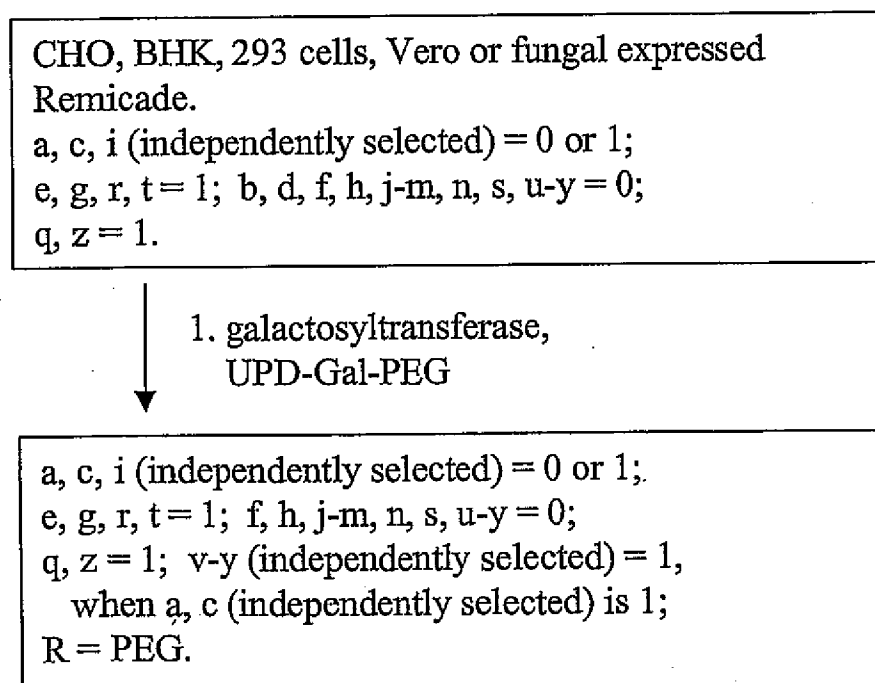   when a, c (independently selected) is 1;
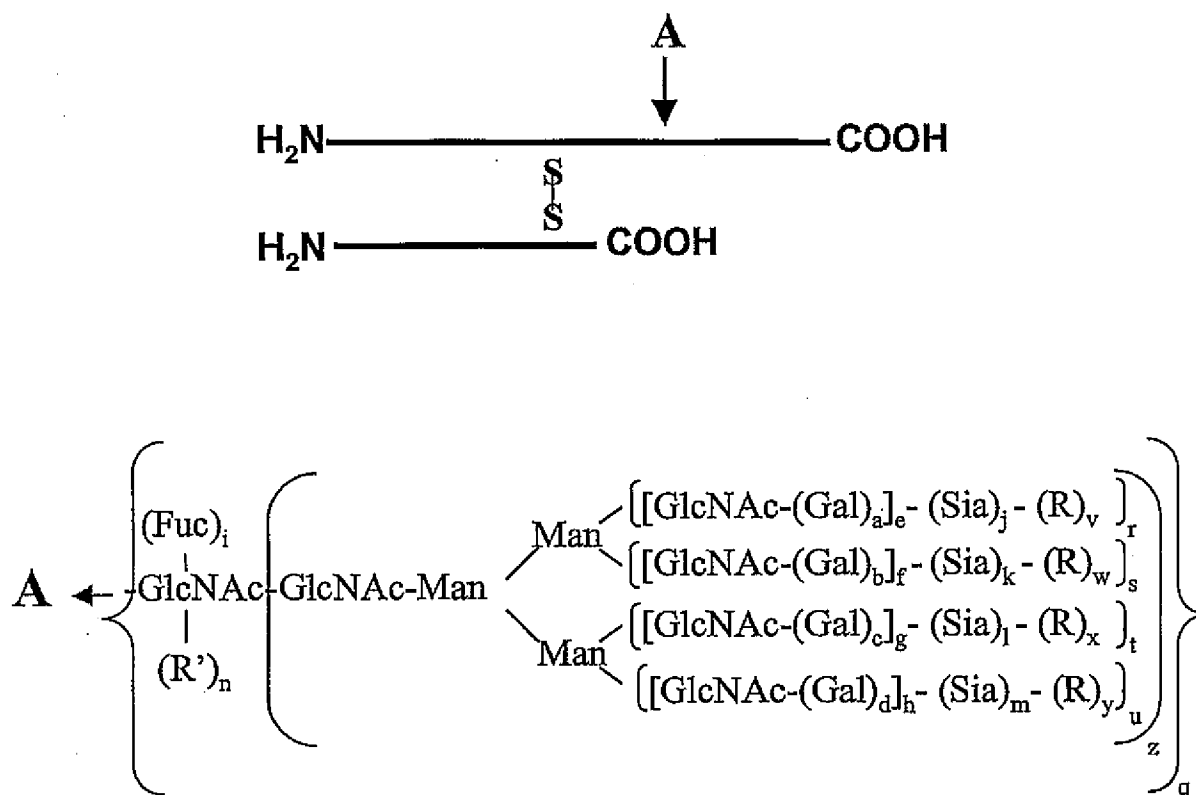R = PEG.

FIG. 49C

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Fungi expressed Remicade.
e, g, i, r, t (independently selected) = 0 or 1;
a-d, f, h, j-m, n, s, u-y = 0; q, z = 1.

1. Endo-H
2. Galactosyltransferase, UDP-Gal
3.. CMP-SA-radioisotope complex, ST3Gal3

a-m, r-z = 0; q, n = 1;
R' = -Gal-Sia-radioisotope complex.

FIG. 49D

$$A$$

$$H_2N \underline{} \overset{\displaystyle A}{\underset{\displaystyle S}{\downarrow}} \underline{} COOH$$

$$\underset{S}{\overset{S}{|}}$$

$$H_2N \underline{} COOH$$

$$A \leftarrow \left\{ \begin{array}{c} (Fuc)_i \\ | \\ GlcNAc-GlcNAc-Man \\ | \\ (R')_n \end{array} \left\langle \begin{array}{c} Man \overset{\displaystyle \left[[GlcNAc-(Gal)_a]_e - (Sia)_j - (R)_v \right]_r}{\underset{\displaystyle \left[[GlcNAc-(Gal)_b]_f - (Sia)_k - (R)_w \right]_s}{}} \\ Man \overset{\displaystyle \left[[GlcNAc-(Gal)_c]_g - (Sia)_l - (R)_x \right]_t}{\underset{\displaystyle \left[[GlcNAc-(Gal)_d]_h - (Sia)_m - (R)_y \right]_u}{}} \end{array} \right\rangle_z \right\}_q$$

a-d, i, q-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 4.
j-m (independently selected) = 0 or 1.
n, v-y = 0; z = 0 or 1;
R = modifying group, mannose, oligo-mannose;
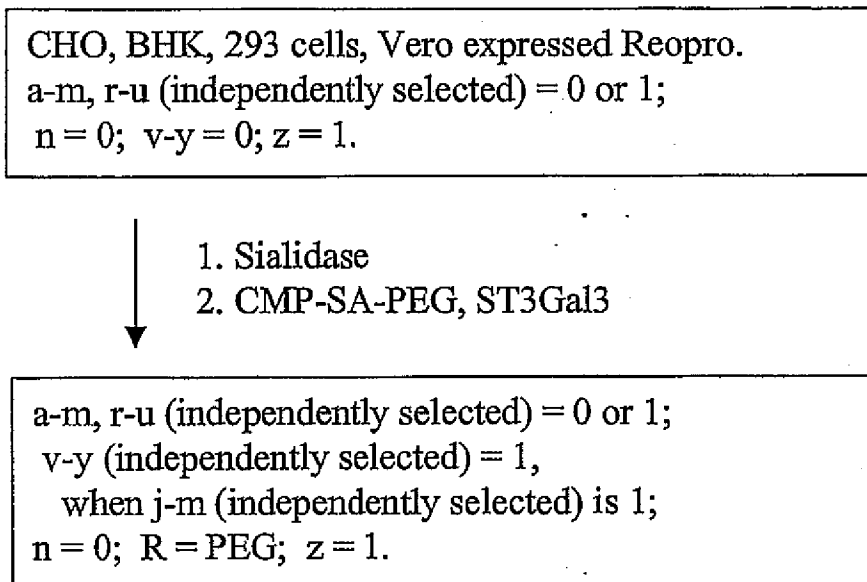R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 50A

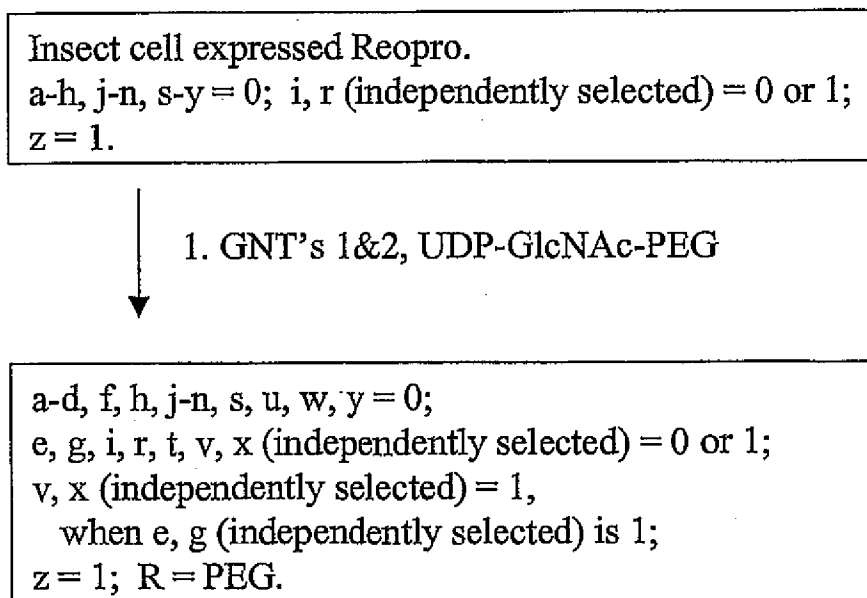CHO, BHK, 293 cells, Vero expressed Reopro.
a-m, r-u (independently selected) = 0 or 1;
n = 0; v-y = 0; z = 1.

↓ 1. Sialidase
    2. CMP-SA-PEG, ST3Gal3

a-m, r-u (independently selected) = 0 or 1;
v-y (independently selected) = 1,
    when j-m (independently selected) is 1;
n = 0; R = PEG; z = 1.

FIG. 50B

Insect cell expressed Reopro.
a-h, j-n, s-y = 0; i, r (independently selected) = 0 or 1;
z = 1.

↓ 1. GNT's 1&2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, v, x (independently selected) = 0 or 1;
v, x (independently selected) = 1,
    when e, g (independently selected) is 1;
z = 1; R = PEG.

FIG. 50C

Yeast expressed Reopro.
a-n = 0; r-y (independently selected) = 0 to 1;
z = 1;
R (branched or linear) = Man, oligomannose or
polysaccharide.

1. Endo-H
2. Galactosyltransferase, UDP-Gal-PEG

a-m, r-z = 0; n = 1; R' = -Gal-PEG.

FIG. 50D
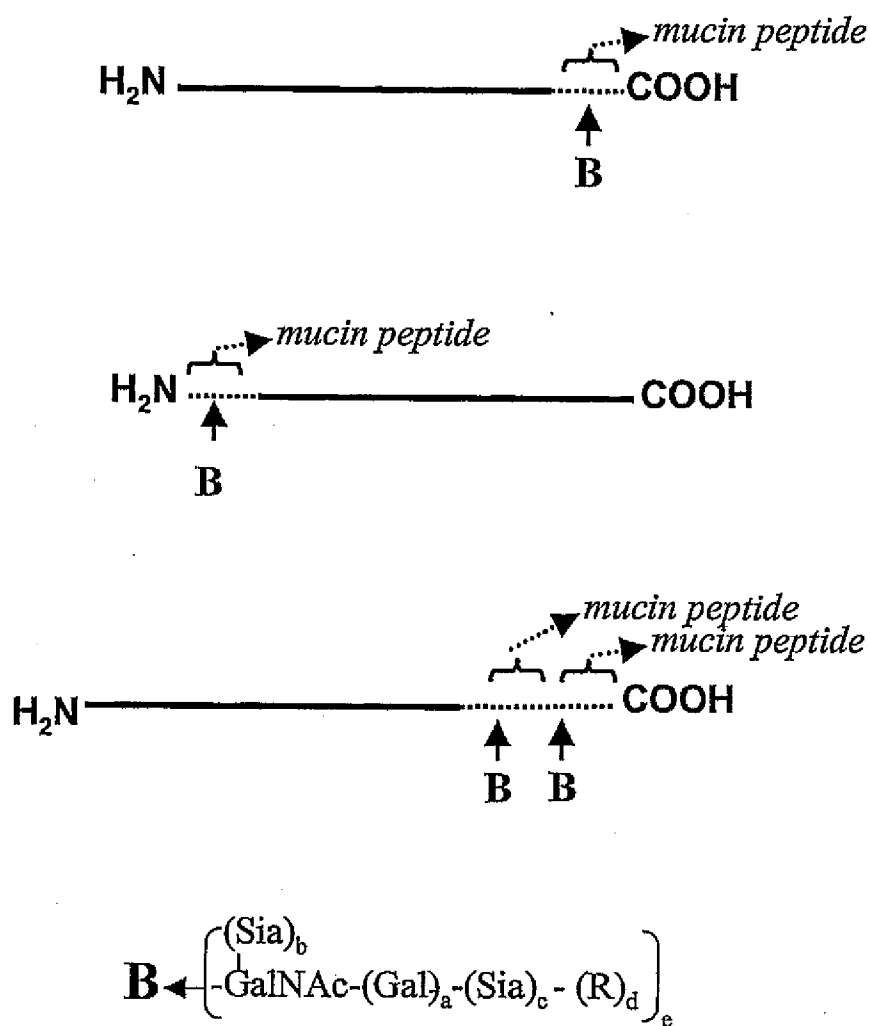
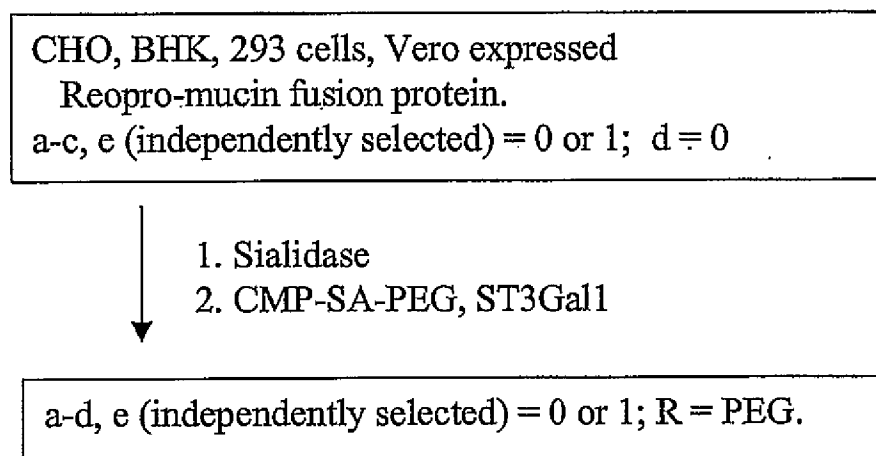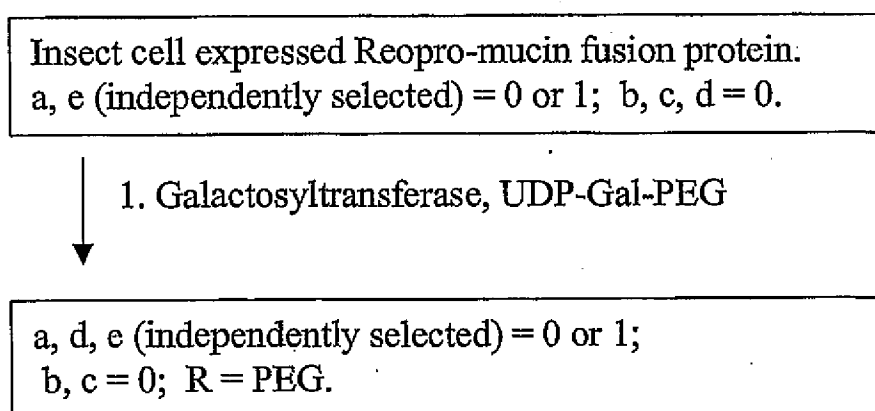a-c, e (independently selected) = 0 or 1;
d = 0; R = polymer

FIG. 50E

CHO, BHK, 293 cells, Vero expressed
  Reopro-mucin fusion protein.
a-c, e (independently selected) = 0 or 1; d = 0

1. Sialidase
2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

FIG. 50F

Insect cell expressed Reopro-mucin fusion protein.
a, e (independently selected) = 0 or 1; b, c, d = 0.

1. Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1;
  b, c = 0; R = PEG.

FIG. 50G

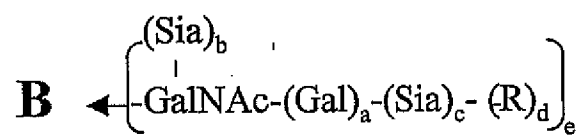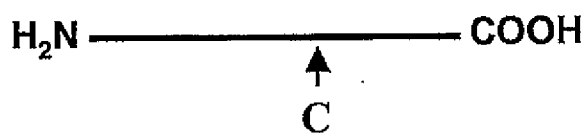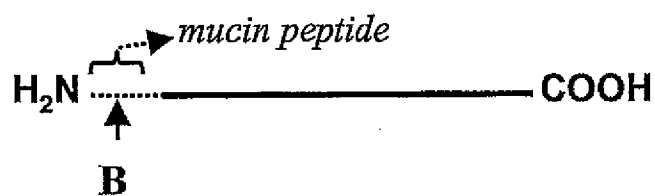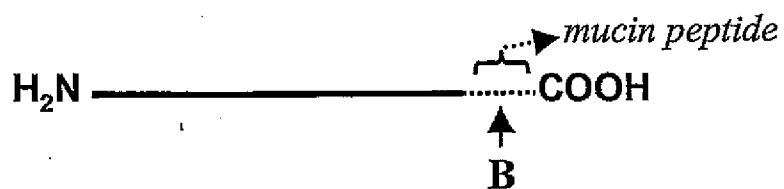E. coli expressed Reopro-mucin fusion protein.
a-e = 0.

1. GalNAc Transferase, UDP-GalNAc
2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1;
a, b = 0; R = PEG.

FIG. 50H

$$H_2N \longrightarrow \overbrace{}^{\nearrow mucin\ peptide} COOH$$
$$\underset{B}{\uparrow}$$

$$\overset{\nearrow mucin\ peptide}{\overbrace{}} $$
$$H_2N \cdots\cdots\cdots \longrightarrow COOH$$
$$\underset{B}{\uparrow}$$

$$H_2N \longrightarrow COOH$$
$$\underset{C}{\uparrow}$$

$$\mathbf{B} \longleftarrow \left[\begin{array}{c} (Sia)_b \\ | \\ \text{-GalNAc-(Gal)}_a\text{-(Sia)}_c\text{- (R)}_d \end{array} \right]_e$$

$$\mathbf{C} \longleftarrow (R')_n$$
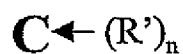
a-c, e (independently selected) = 0 or 1;
d = 0; R = polymer, linker.

FIG. 50I

E. coli expressed Reopro-mucin fusion protein.
a-e, n = 0.

1. GalNAc Transferase,
   UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1;
a-c, n = 0; R = PEG.

FIG. 50J

E. coli expressed Reopro-mucin fusion protein.
a-e, n = 0.

1. GalNAc Transferase,
   UDP-GalNAc-linker-SA-CMP
2. ST3Gal3, asialo-transferrin
3. CMP-SA, ST3Gal3

d, e (independently selected) = 0 or 1;
a-c, n = 0; R = linker-transferrin.
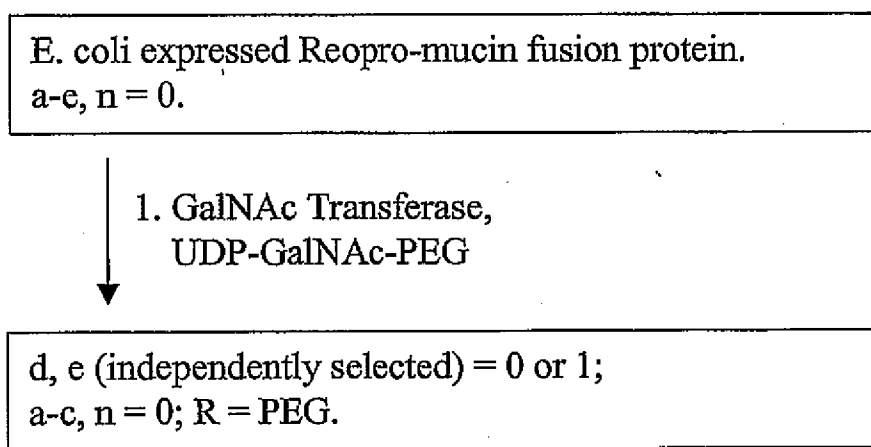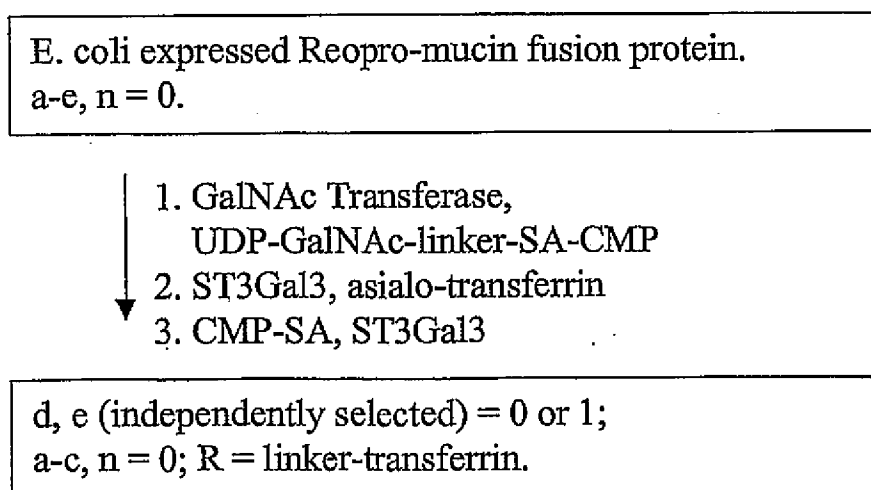
FIG. 50K

E. coli expressed Reopro(N)—no mucin peptide.
a-e, n = 0.

1. NHS-CO-linker-SA-CMP
2. ST3Gal3, asialo-transferrin
3. CMP-SA, ST3Gal3

a-e = 0; n = 1; R' = linker-transferrin.

FIG. 50L